GEORGIA INSTITUTE OF TECHNOLOGY — OFFICE OF CONTRACT ADMINISTRATION

PROJECT ADMINISTRATION DATA SHEET

[x] ORIGINAL [ ] REVISION NO. _____

Project No. E-20-616 DATE 10/27/81

Project Director: Dr. E. S. K. Chian School/~~Lab~~ Civil Eng.

Sponsor: Environmental Protection Agency, Cincinnati, OH

Type Agreement: Contract No. 68-03-3094

Award Period: From 9/15/81 To 9/14/83 (Performance) 9/14/83 (Reports)

Sponsor Amount: $69,746 (incrementally funded at $60,000) thru 4/15/83) Contracted through:

Cost Sharing: _____ GTRI/~~GK~~

Title: Comparison of High Molecular Weight Organics Isolated From Drinking Water in Five Cities

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Defense Priority Rating: N/A Security Classification: N/A

RESTRICTIONS

See Attached _____ Supplemental Information Sheet for Additional Requirements.

Travel: Foreign travel must have prior approval – Contact OCA in each case. Domestic travel requires sponsor approval where total will exceed greater of $500 or 125% of approved proposal budget category.

Equipment: Title vests with Government, except that title to items costing less than $1,000 shall vest with GIT upon acquisition provided that prior approval received from C.O.

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SPONSORED PROJECT TERMINATION/CLOSEOUT SHEET

Date April 20, 1984

Project No. E-20-616 School ~~XXXX~~ Civil Engineering

Includes Subproject No.(s) E-27-611

Project Director(s) Dr. E.S.K. Chian GTRI / ~~XXX~~

Sponsor Enviromental Protection Agency, Cincinnati, OH

Title "Comparison of High Molecular Weight Organics Isolated From Drinking Water in Five Cities"

Effective Completion Date: 9/14/83 (Performance) 9/14/83 (Reports)

Grant/Contract Closeout Actions Remaining:

- [ ] None
- [ ] Final Invoice or Final Fiscal Report
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GEORGIA INSTITUTE OF TECHNOLOGY OFFICE OF CONTRACT ADMINISTRATION

SPONSORED PROJECT TERMINATION/CLOSEOUT SHEET

Date April 20, 1984

Project No. E-27-611 Sub under E-20-616/Chian/CE School/~~Unit~~ Textile Engr.

Includes Subproject No.(s)

Project Director(s) Wayne Tincher GTRI / ~~Unit~~

Sponsor Enviromental Protection Agency, Cincinnati, OH

Title "Comparison of High Molecular Weight Organics Isolated from Drinking Water in Five Cities."

Effective Completion Date: 9/15/81 (Performance) 9/14/83 (Reports)

Grant/Contract Closeout Actions Remaining:

- ☐ None
- ☐ Final Invoice or Final Fiscal Report
- ☐ Closing Documents
- ☐ Final Report of Inventions
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COMPARISON OF HIGH MOLECULAR WEIGHT ORGANICS ISOLATED FROM DRINKING WATER IN FIVE CITIES

QUARTERLY REPORT
December 1981

by

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Contract No. 68-03-3094

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INDEX

	Page
Introduction	1
Fractionation	2
UV and Fluorescence Spectra	7
Infrared Absorption	17
Elemental Analysis	21
X-ray Scattering	23
Size exclusion chromatography or ultrafiltration	28
Thermal gravimetric analysis	30
Estimation of acid groups	30
Summary	31
Future plan	36

INTRODUCTION

The report presents the work performed in the direction of analyzing ten samples, supplied by EPA, and derived from the drinking water of 5 cities. The samples are listed in Table 1. Sample No. 1 (Miami 1A) is a prototype of the acetone soluble fraction. The other 10 are the samples to be investigated fully.

TABLE 1

List of samples received:

1. Miami 1A (acetone soluble fraction, 130 mg)
2. Miami 1B (XAD eluate)
3. Miami 1A (CA and Nylon)
4. Miami a XAD
5. New Orleans Ia
6. New Orleans 2 XAD
7. Philly 1
8. Ottumwa
9. OZX - 60%
10. SZX - Seattle
11. P2X - Original

Our tasks on these samples are listed sequentially:

1. Fractionation of the sample according to solubility in hexane, ether and acetone will result in 4 fractions:

(i) Soluble in hexane

(ii) Soluble in ether

(iii) Soluble in acetone

(iv) Insoluble

Weights of the starting gross sample and these 4 fractions will be determined.

All subsequent analytical work as itemized below will be done only on the acetone soluble fraction.

2. Recording UV, IR and fluorescence absorption spectrum.

3. Elemental analysis.

4. Molecular weight determination by X-ray scattering.

5. Molecular weight determination by size exclusion chromatography/ ultrafiltration.

6. Thermal gravimetric analysis.

7. Determination of phenol and carboxyl group content.

1. Fractionation

The list of apparatus and/or assembly of apparatus as suggested by EPA have been acquired and assembled. Fractionation was done first on a river water humus sample to verify the reproducibility of our fractionation experiments. They were found satisfacoty, and 1-g aliquots of two samples have been fractionated. The results are presented in the following pages.

Verification of Reproducibity Of Fractionation Experiments

Sample -Riverswamp Humus (A-6).

Fractionation was done according to the procedure supplied by EPA. The results are as follows:

Expt. No.	Wt. of Sample	FRACTIONS									
		Hexane		Ether		Acetone		Insoluble		Total Recovery	
		Wt	%	Wt	%	Wt	%	Wt	%	Wt	%
1	100	4.00	4.0	2.45	2.5	8.00	8.0	91.00	91.0	105.45	105.5
2	100	3.46	3.5	2.13	2.1	7.57	7.6	95.99	96.0	99.15	99.2
3	1000	6.14	0.6	18.53	1.9	33.20	3.3	912.15	91.2	970.02	97.0

Mean Total Recovery = 100.6%

Standard deviation = 4.4

Coefficient of variability = 4.4%

Replicate Expts. 1 and 2 establish the reproducibility of the experimental procedure. A 5.3% reduction in sample recovery is accompanied with 10-fold (1000%) increase in sample weight. This reduction is distributed mainly between the hexane and acetone fraction.

All weights are in mg.

	1 Δ Recovery	2 ΔSample Wt	3* % Change in recovery with increase in sample wt
Hexane	-3.2	+900	-.36
Ether	-0.4	+900	-.04
Acetone	-4.5	+900	-.50
Insoluble	-2.3	+900	-.25

*100% change in sample weight will produce a change of 0.5% in the recovery of acetone fraction.

Column 3 represents percentage change in recovery with change in sample weight from 100 to 1000 mg. The expression is

$$\frac{\Delta \text{Recovery}}{\Delta \text{Sample Wt}} \times 100$$

As this expression is uniformly low (≤ 0.5), it is expedient to process 1000 mg of sample instead of 100 mg.

Sample MIAMI 1B

Fractionation into 4 parts by solubility in different solvents

		Percent
Weight of sample	1.04245 g	100
Fraction soluble in hexane	44.74 mg	4.29
Fraction soluble in ether	354.34 mg	33.99
Fraction soluble in acetone	498.75 mg	47.84
Insoluble fraction	4.36 mg	0.42
Total Recovery	902.19 mg	86.55

Sample 02X-60% 1-11-79
Ottumwa A2-XAD

Fractionation into 4 parts by solubility
in different solvents

		Percent
Weight of sample	1.07180 g	100
Fraction soluble in hexane	25.3 mg	2.36
Fraction soluble in ether	191.9 mg	17.90
Fraction soluble in acetone	198.3 mg	18.50
Insoluble fraction	74.27 mg	6.93
Total Recovery	489.77 mg	45.70

2. UV and Fluorescence Spectra

These spectra were obtained from a methanolic solution of the samples. The graphs are presented with titles and relevant informations (Figures 1 through 9). Holmium oxide filter was used to verify absorption maxima at the known wavelengths (Fig. 1). The Rayleigh and Raman peaks were scanned (Fig. 6) to verify these wavelengths both graphically and by noting the maximum meter reading directly to verify wavelength in the fluorescence spectrophotometer.

The emmission spectra were obtained by setting the excitation wavelength at the excitation maxima and vice-versa. Miami 1A (Fig. 7)and Ottumwa (Fig. 9) show the same abosrption pattern while Miami 1B (Fig. 8) differs. A sample of riverwater humus shows emmission and excitation maxima at 460 and 365 nm respectively. These values are identical to those for Miami 1B.

UV absorption of the 3 samples (Fig. 2,3,4) are virtually the same.

Organic compounds show some absorption near 210 nm due to $n \longrightarrow \sigma^*$ transition if the molecule contains O, N, S or halogen atoms. However, our compounds do not exhibit the typical end absorption that increases towards shorter wavelength. α,β - Unsaturated acids, esters and amides generally show a high intensity absorption peak in the region 205-225. (ε 10,000 - 20,000). Assuming the molecular weight of the macromolecule to be 1000, ε is 17,000 (for Miami 1B) and 21,000 (for Ottumwa). It is an indication that $\alpha\beta$ unsaturated acids substructures may be present.

λ_{max} (nm)			
Found	Should be	Difference	Tolerance
279.1	279.3	-0.2	±1.0
287.3	287.6	-0.3	±1.0

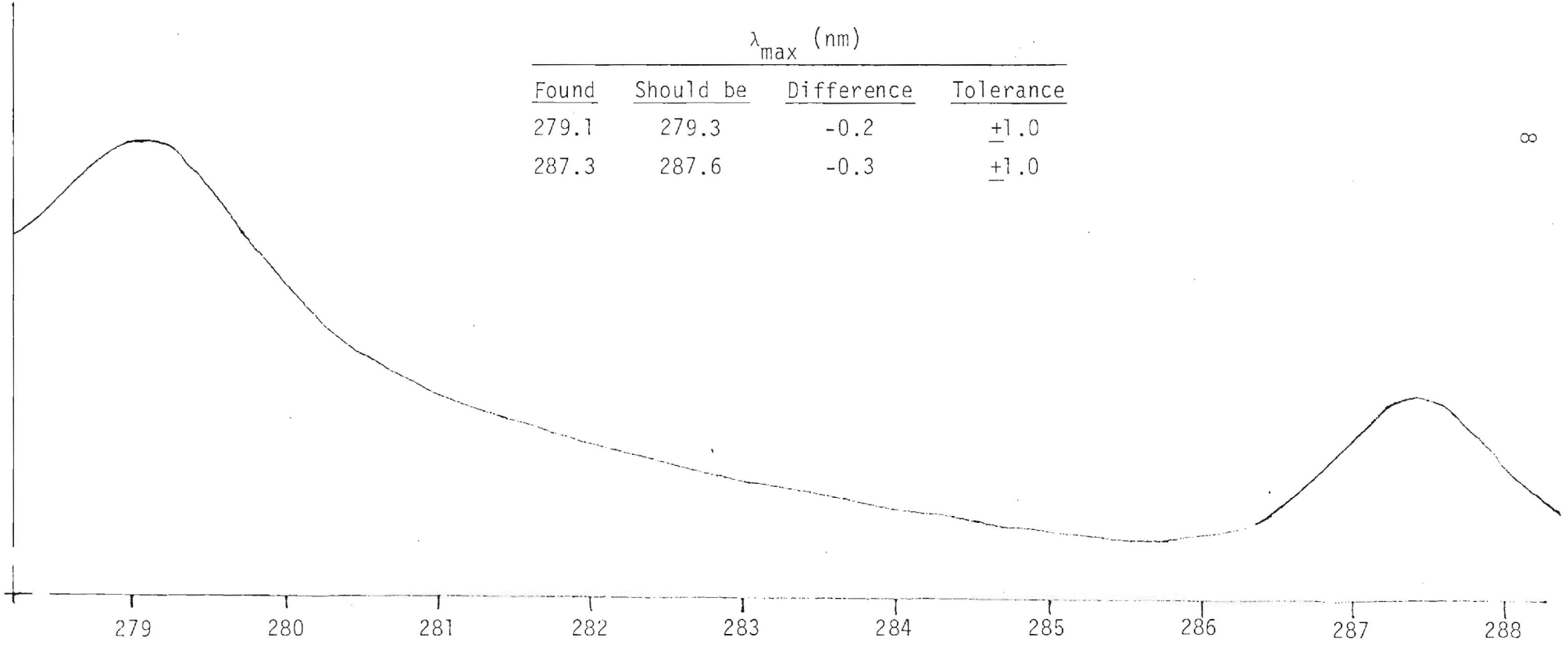


Figure 1. UV absorption of holmium oxide filter.

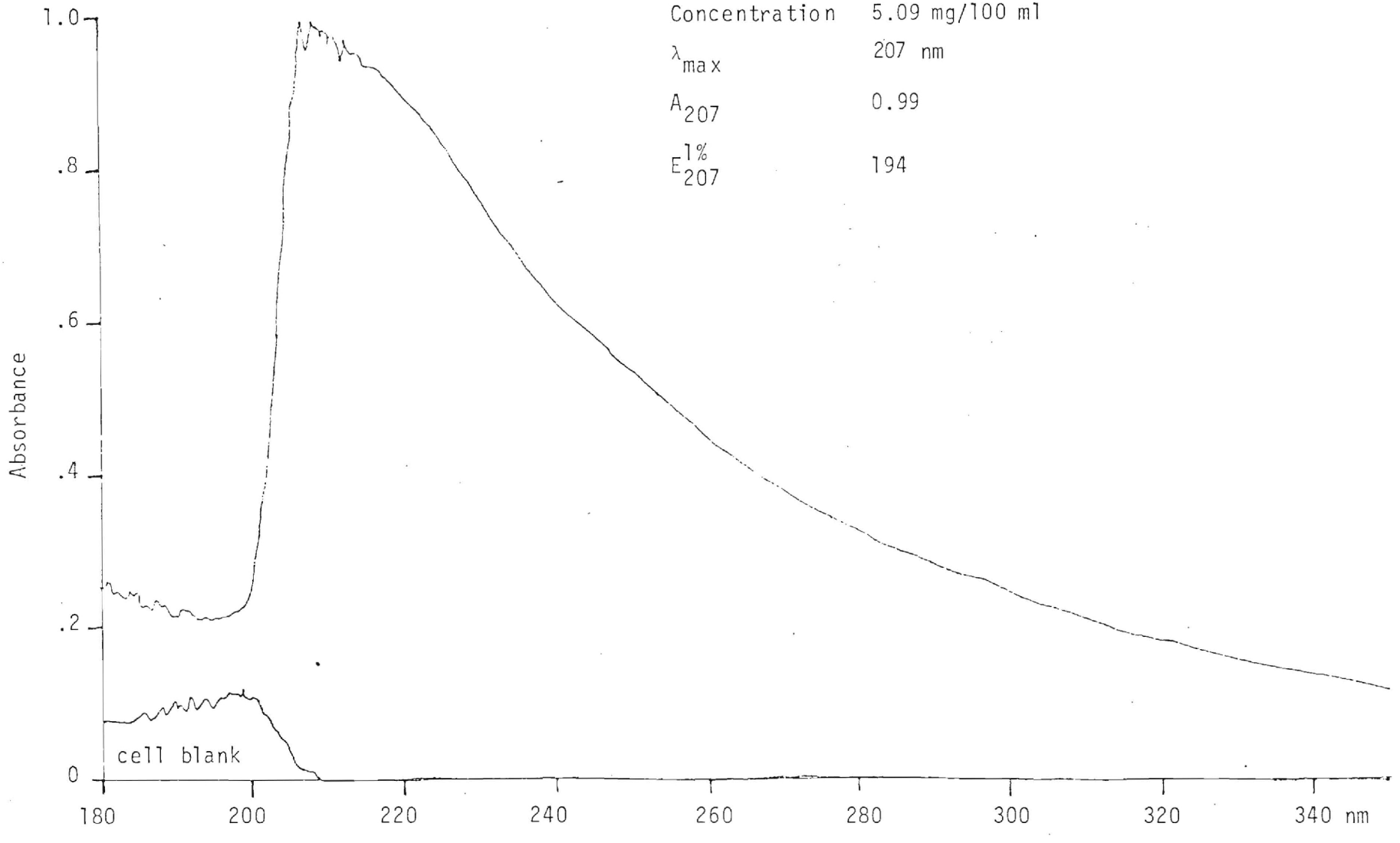


Figure 2. UV absorption spectrum of Miami 1A.

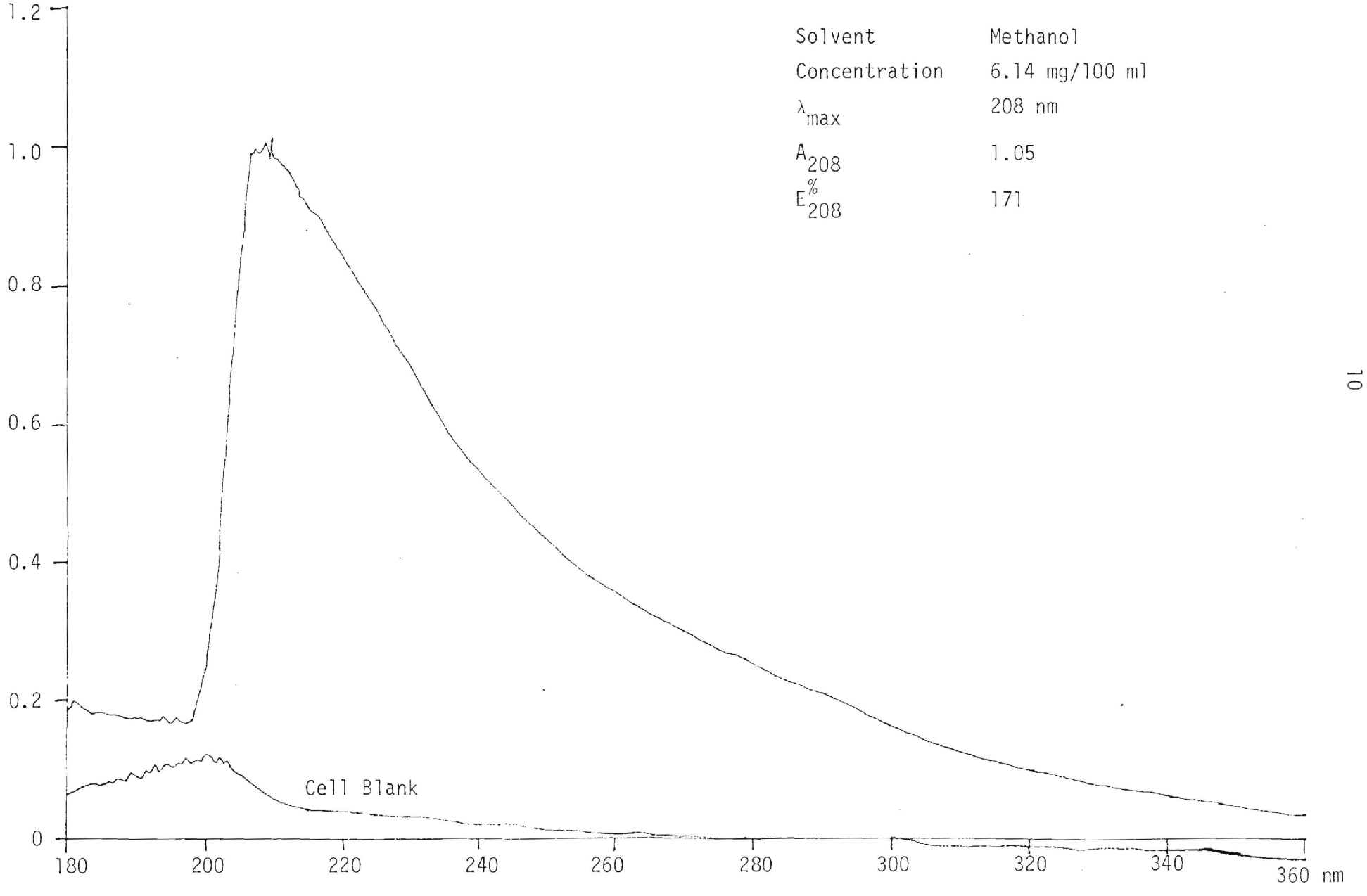


Figure 3. UV absorption of Miami 1B.

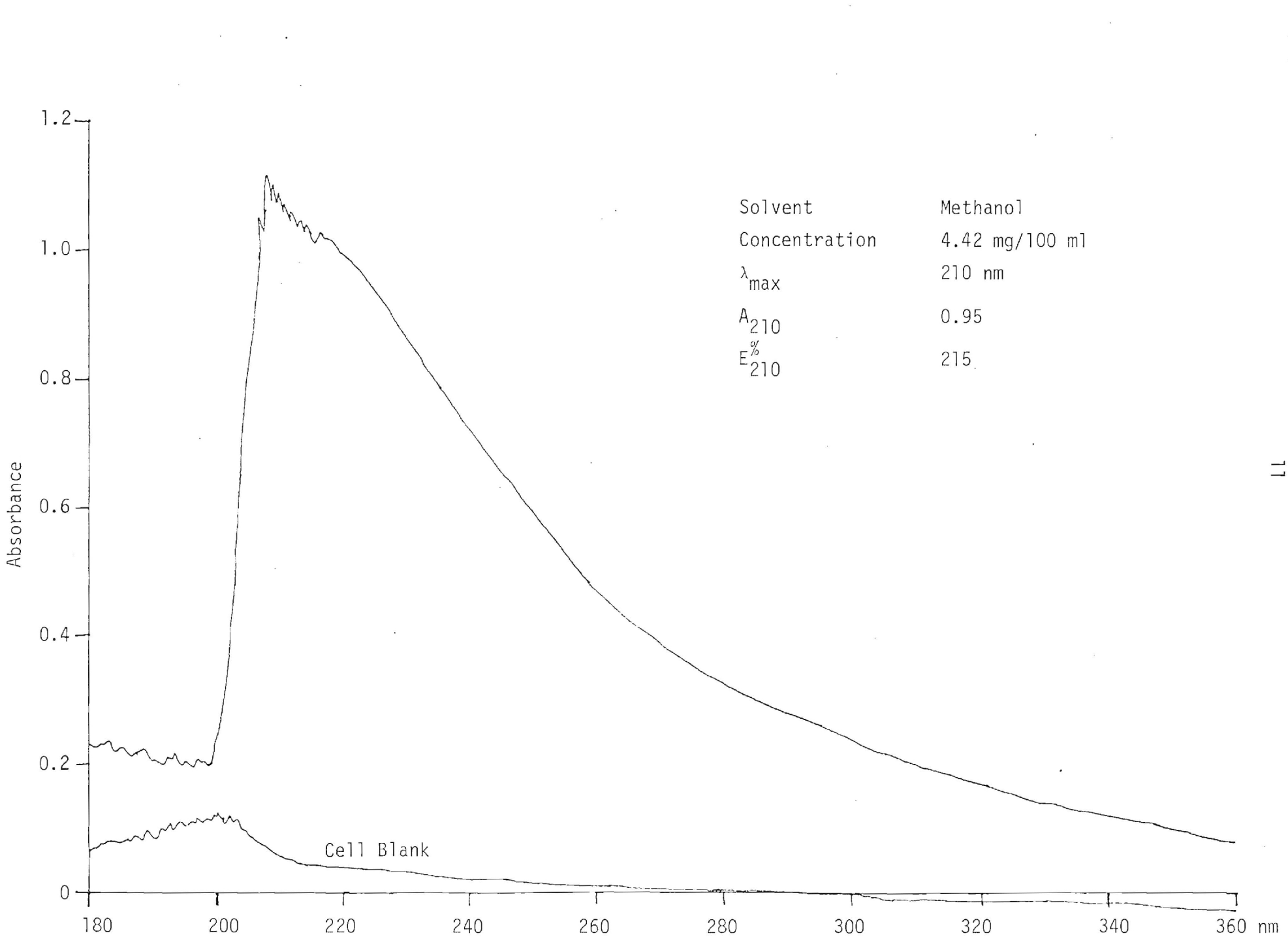


Figure 4 . UV absorption of Ottumwa A2.

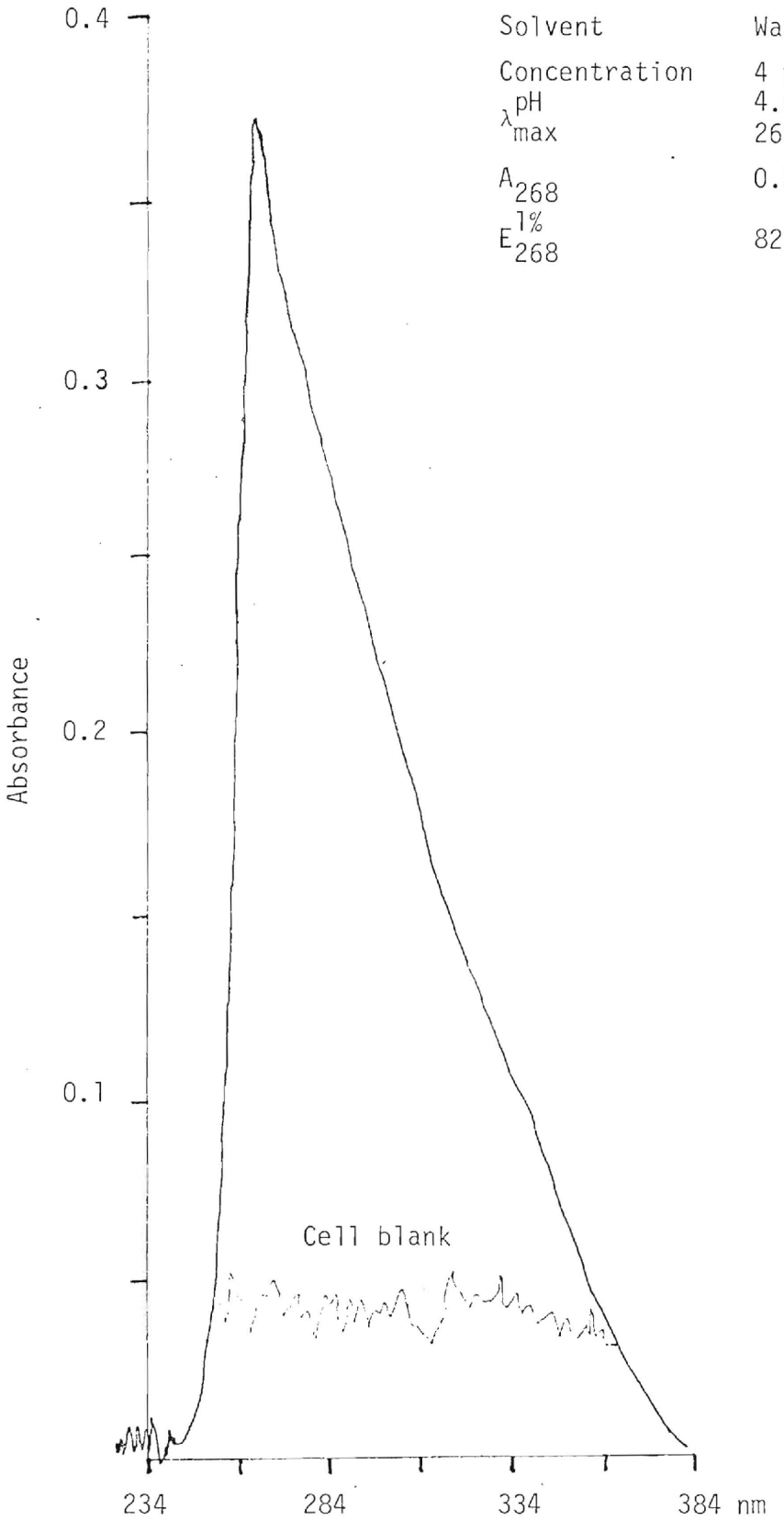


Figure 5 . UV absorption of humus derived from Satilla river.

λ_{Em}

Found	Should be	Differences	Tolerance
418 nm	418 nm	±0	5 nm

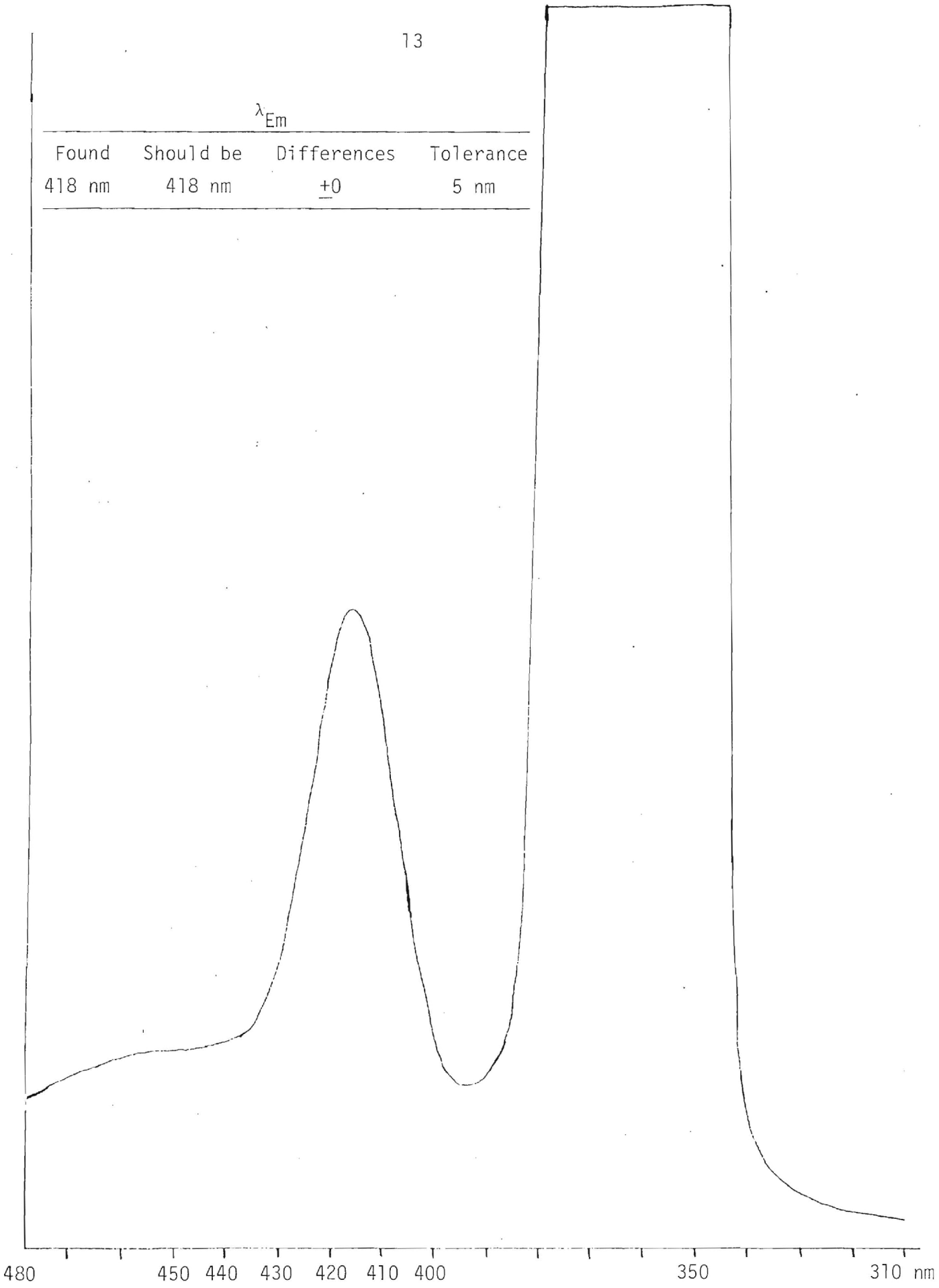


Figure 6. Emission spectrum of water at $\lambda_{excitation}$ 365 nm.

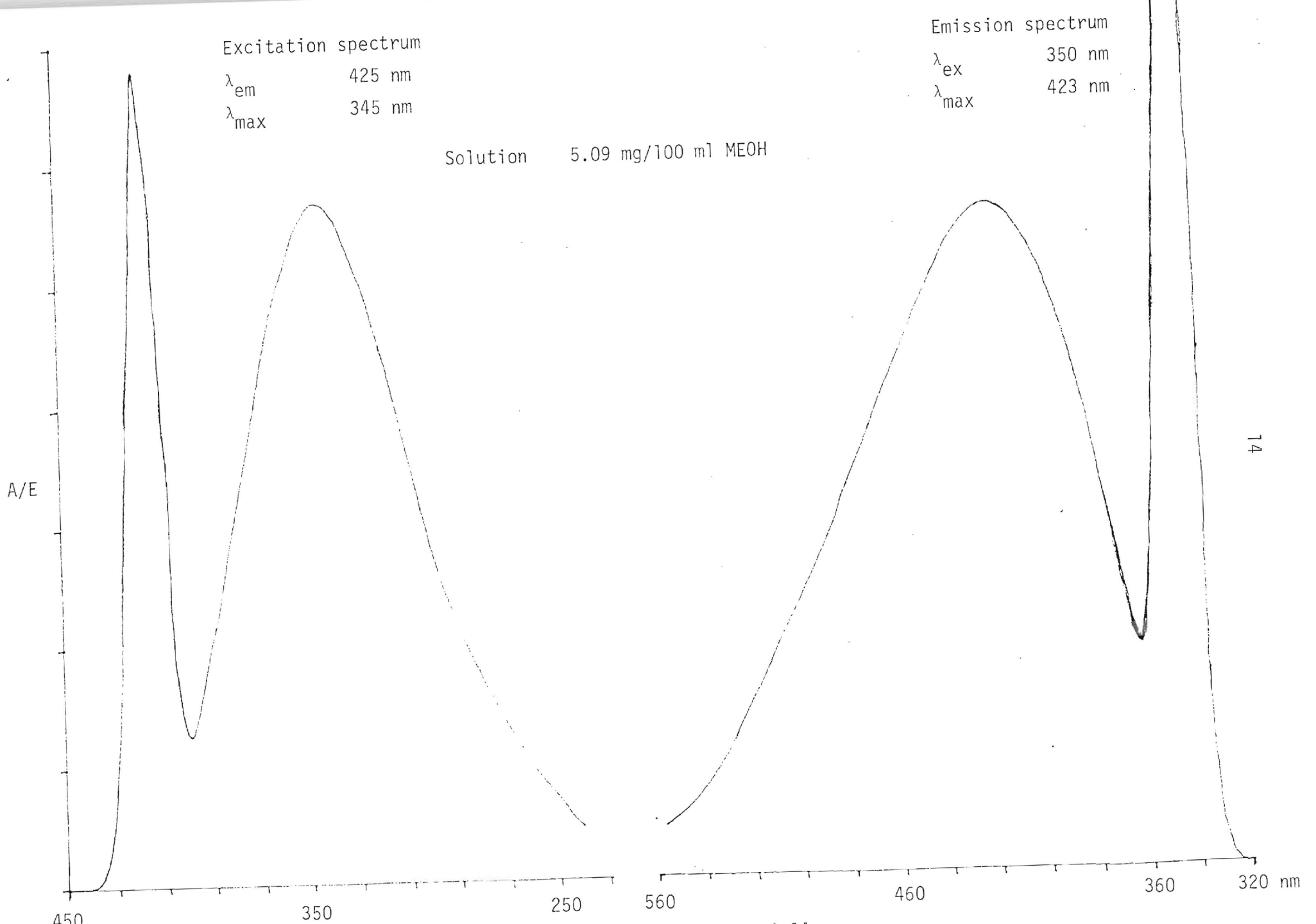


Figure 7 . Fluorescence spectra of acetone soluble fraction of Miami 1A.

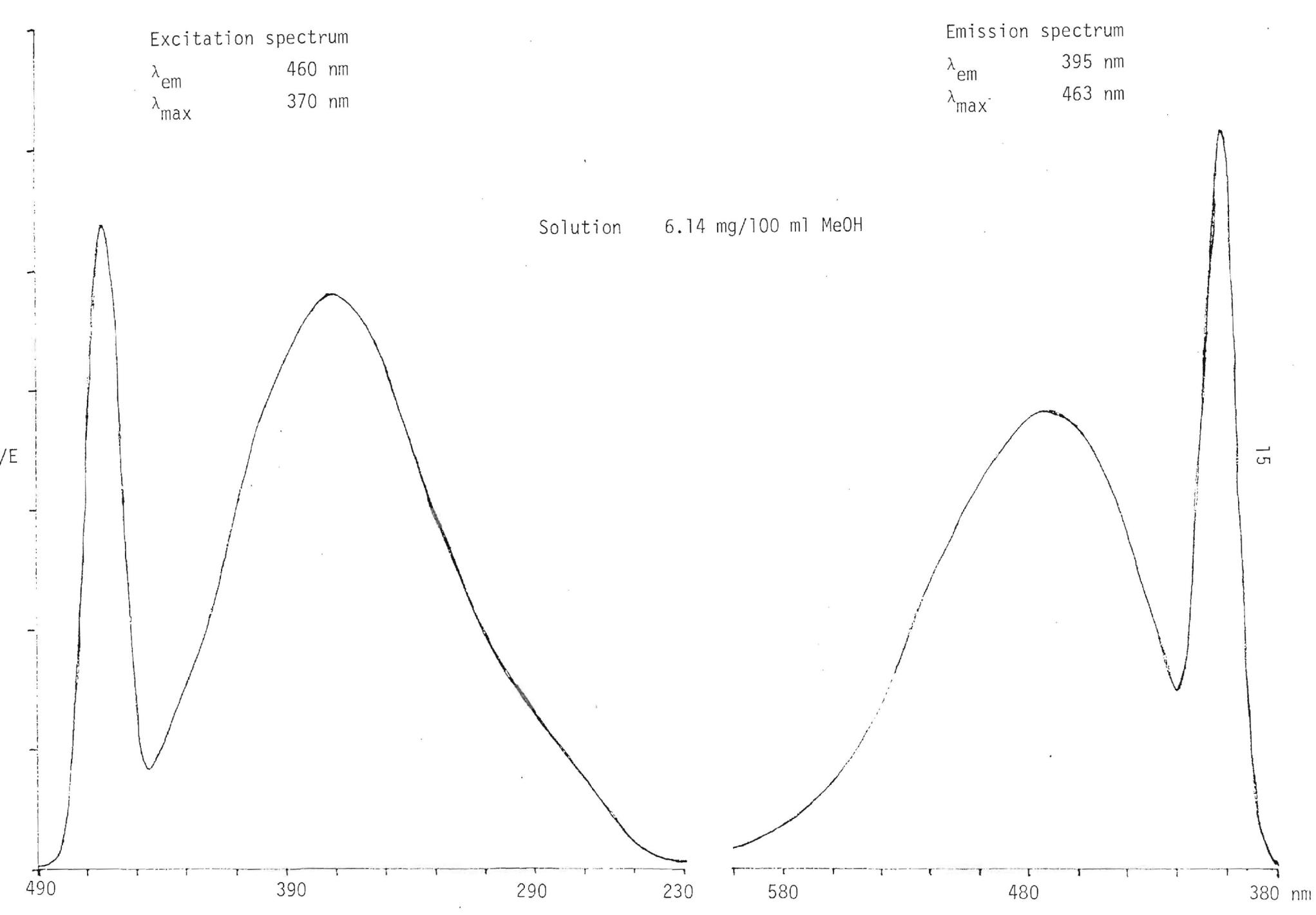


Figure 8. Fluorescence spectra of acetone soluble fraction of Miami 1B.

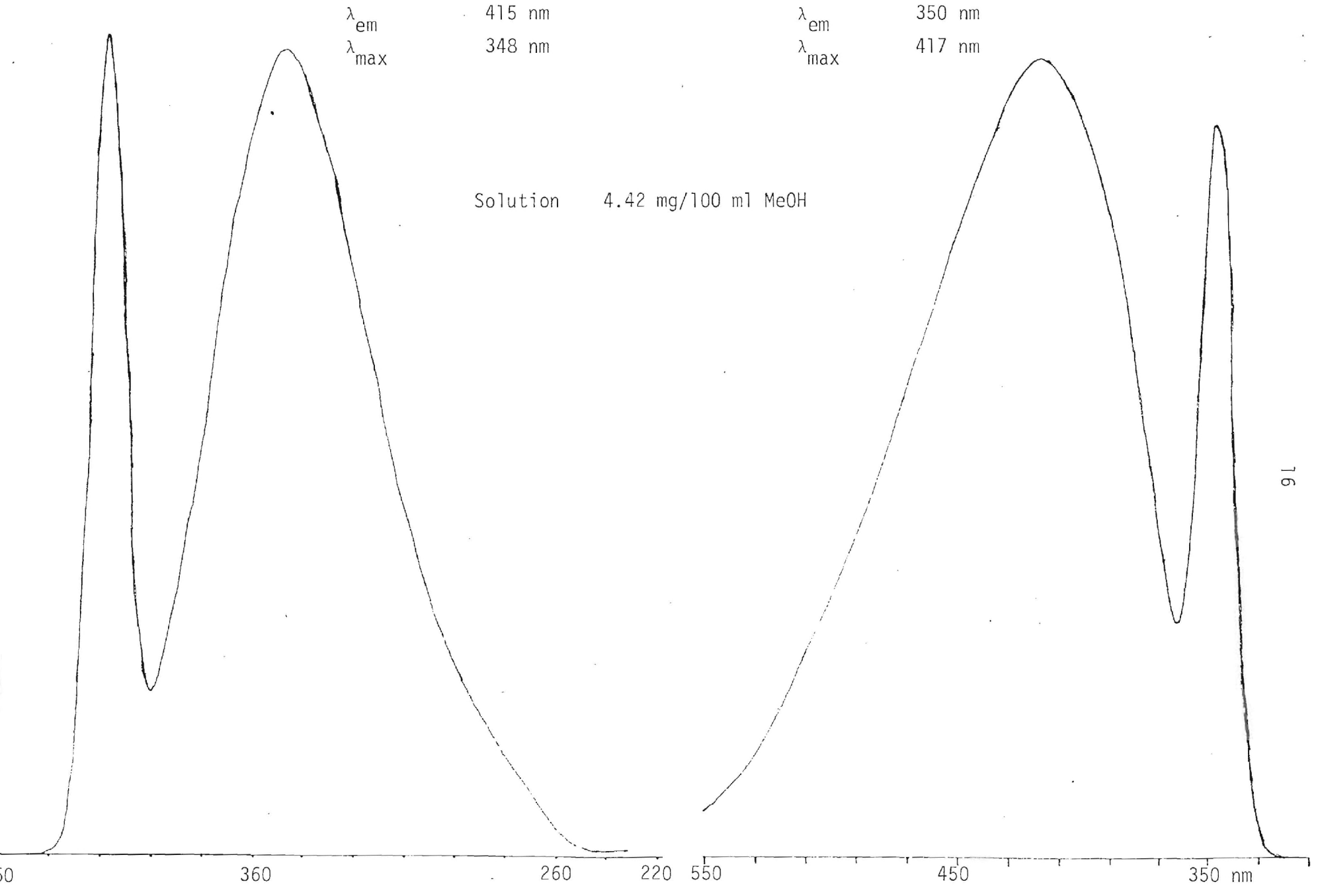


Figure 9. Fluorescence spectra of acetone soluble fraction of Ottumwa.

2. Infrared abosrption

Spectrum of a polystyrene film was recorded to check resolution and wavelength (Fig. 10).

Spectra of two samples were determined as kBr pellets. These are presented in Fig. 11 and Fig. 12. Both these samples show -COOH groups. Ottumwa (Fig. 12) may contain $\alpha\beta$ unsaturated -COOH group (1695 cm^{-1}). -OH groups may be present besides -COOH groups.

Miami 1B	3380 cm^{-1}	-OH
	2950-2930 cm^{-1}	C-H (alkane)
	1710 cm^{-1}	C=O
	The braod -OH absorption indicated -COOH group.	
Ottumwa	3300 cm^{-1}	-OH stretching
	2920-2960 cm^{-1}	C-H stretching (alkane)
	1695 cm^{-1} (broad)	C=O stretching
	The broad -OH absorption indicates -COOH group. In the absence of additional evidence for unsaturations, -COOH group is considered saturated aliphatic.	

CTRUM NO.
E 12·18·81
APLE Polystyrene
URCE
UCTURE
f mm
VENT
NCENTRATION
SE Film
AMENTS
LYST MG

Beckman®
INFRARED
SPECTROPHOTOMETER

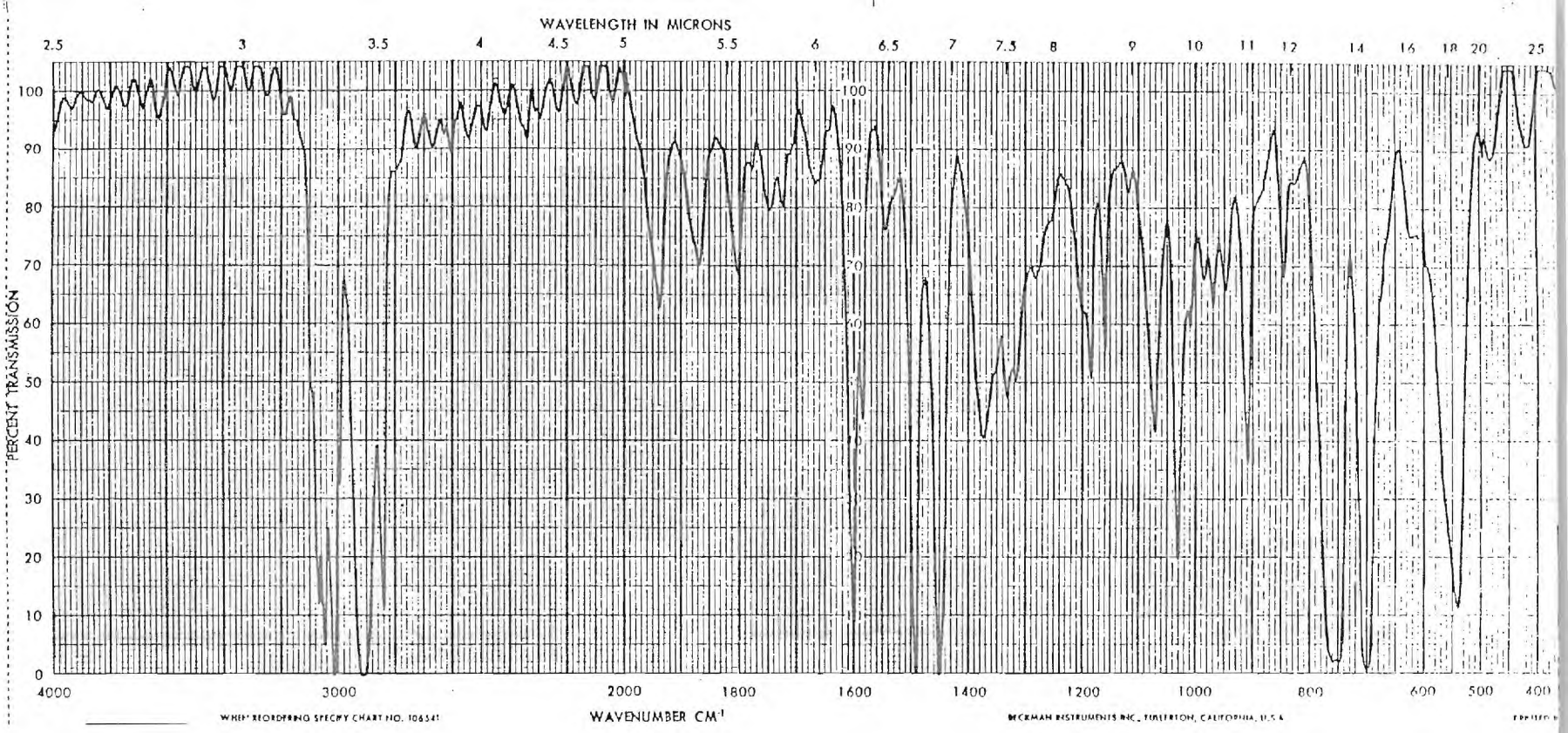


Figure 10.

CTRUM NO. 1
E 12/18/81
PLE MIAMI 1B
AD Eluate
2·17·76 [Lab bk 133, P15]
RCE EPA
UCTURE
Unknown
H mm
VENT
NCENTRATION 2·94 mg/300 mg
SE KBr
AMENTS
ALYST MG

Beckman®
INFRARED
SPECTROPHOTOMETER

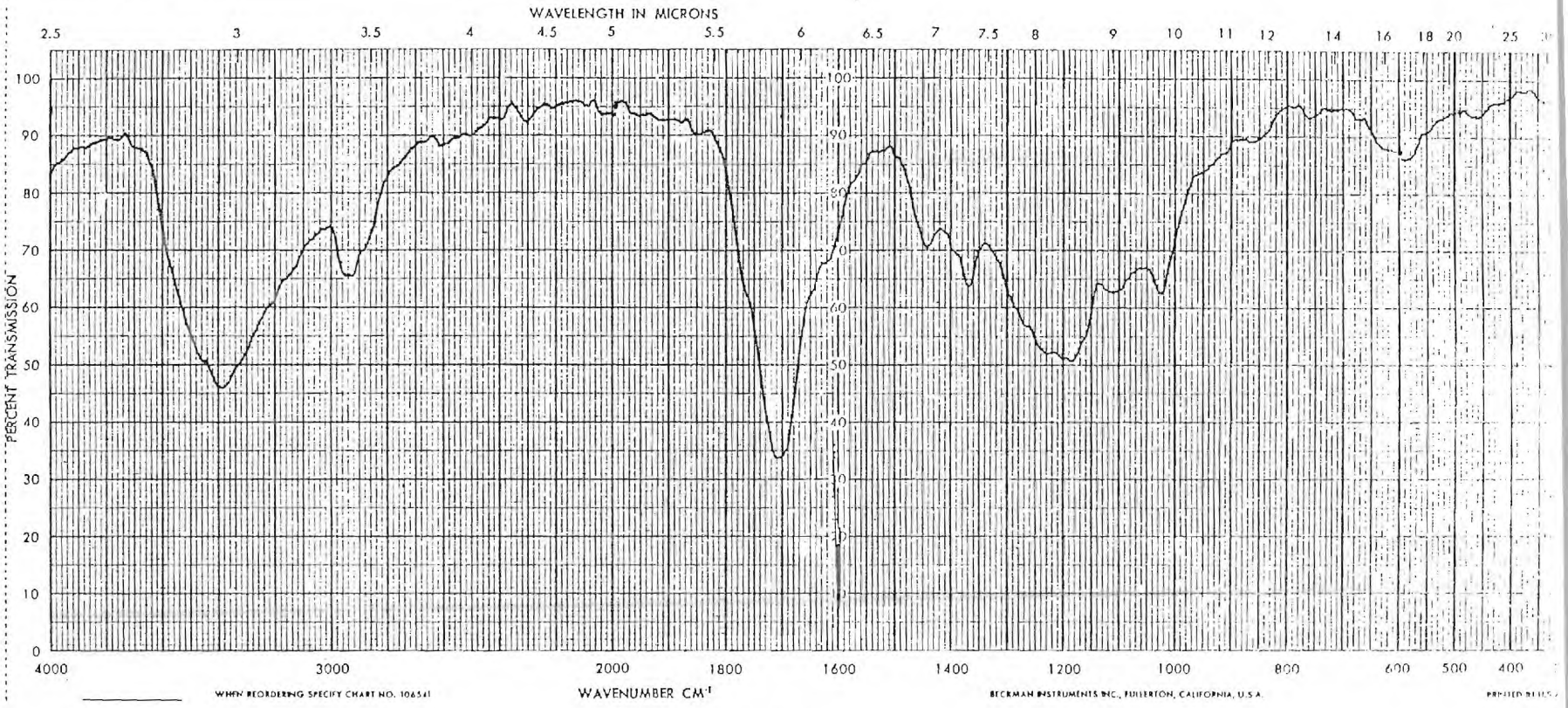


Figure 11.

NO. 2-1
2·18·81
22X- 60%
1 mwa A2.
EPA
nknown
mm
KBr
ATION 1mg/100mg
S
ZG
ckman®
INFRARED
ROPHOTOMETER

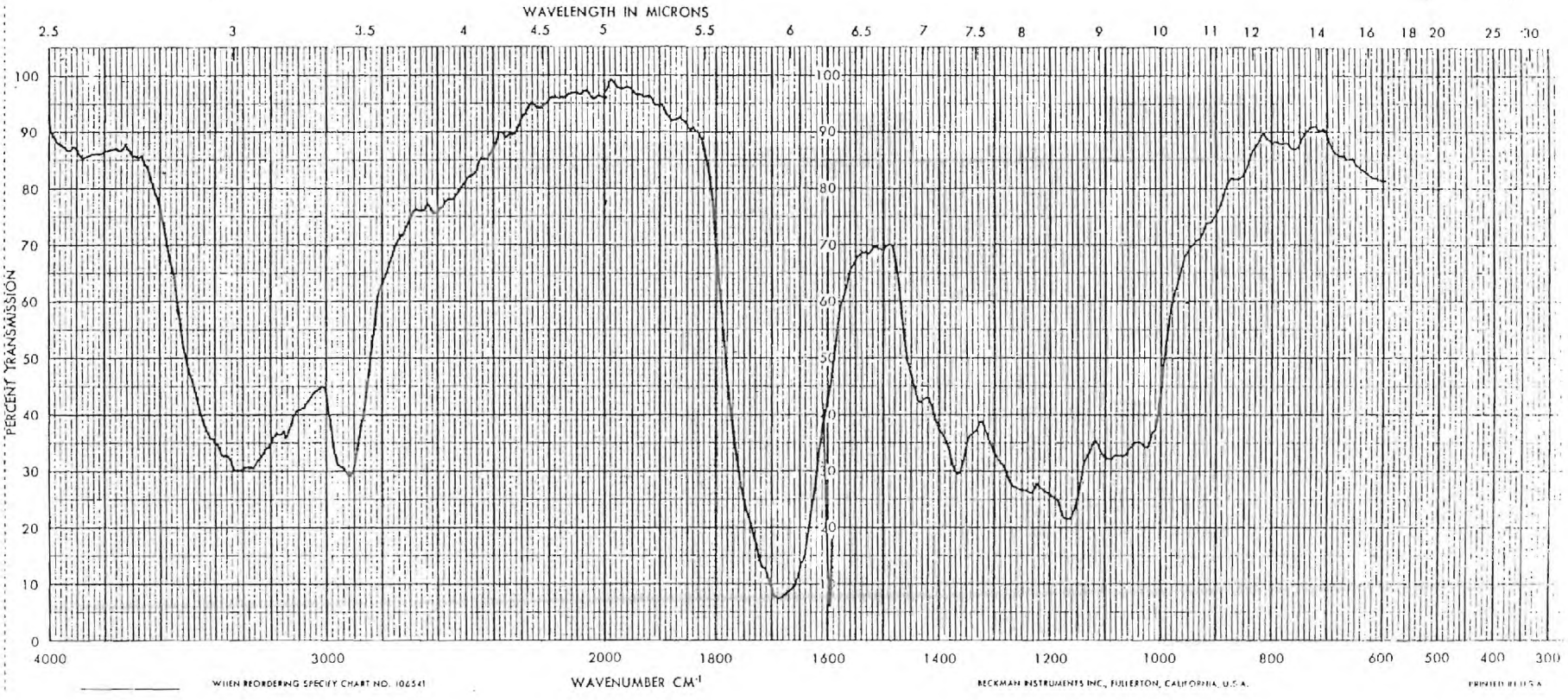


Figure 12.

3. Elemental Analysis

[Done by Atlantic Microlab, Inc.]

Elemental analysis of known samples

1. Acetanilide (C_8H_9NO)

	Theoretical	Found	MAD
C	71.09	71.13	+.04
H	6.71	6.80	+.09
N	10.36	10.34	-.02

2. Chlorobenzoic acid ($C_7H_5O_2Cl$)

Cl	22.65	22.57	-.08

3. Sulfanilamide ($C_6H_8N_2O_2S$)

S	18.62	18.55	-.07

Elemental Analysis

	C	H	N	S	Halogen as Cl or Br	Ash	Cl	Br
Miami 1B	57.51	6.11	2.27	2.07	1.79 or 4.04	0.91	1.45	0.24
Ottumwa	58.31	6.73	2.95	0.94	0 0	0.46	0.30	<0.05

4. Determination of molecular weight (size) by X-ray scattering

Done by Dr. R. L. Wershaw

U. S. Geological Survey

4. X-ray Scattering

Repeated measurements of radius of gyration (Rg) of polyacrylic acid (MW 2000) was recorded between 11.3 - 11.8 Å. From this data, the precision of the experiments is set conservatively at ± 5%.

Rg for octafluorobutane has been reported to be 2.16 Å. X-ray scattering determination of Rg of the same compound varies between 2.17 to 2.18 Å. However, this molecule is small in comparison with the molecules we are dealing with.

X-ray scattering measurement has been completed on one sample. The results are presented below:

Sample	Miami 1A
pH	Radius of gyration (Rg)
11	6.2 Å (MW about 800)
11 (After 1 wk)	8.9 Å
7	8.6 - 8.9 Å
4	23.6 - 13.7 Å

At pH 11, the macromolecules undergo aggregation on keeping for a week. The change in Rg suggests formation of trimers. At pH 7, the aggregation is comparable to what is observed at pH 11 after allowing to stand for 1 week. At pH 4, the degree of aggregation increases enormously and the sample is highly polydisperse.

5. Size exclusion chromatography or ultrafiltration

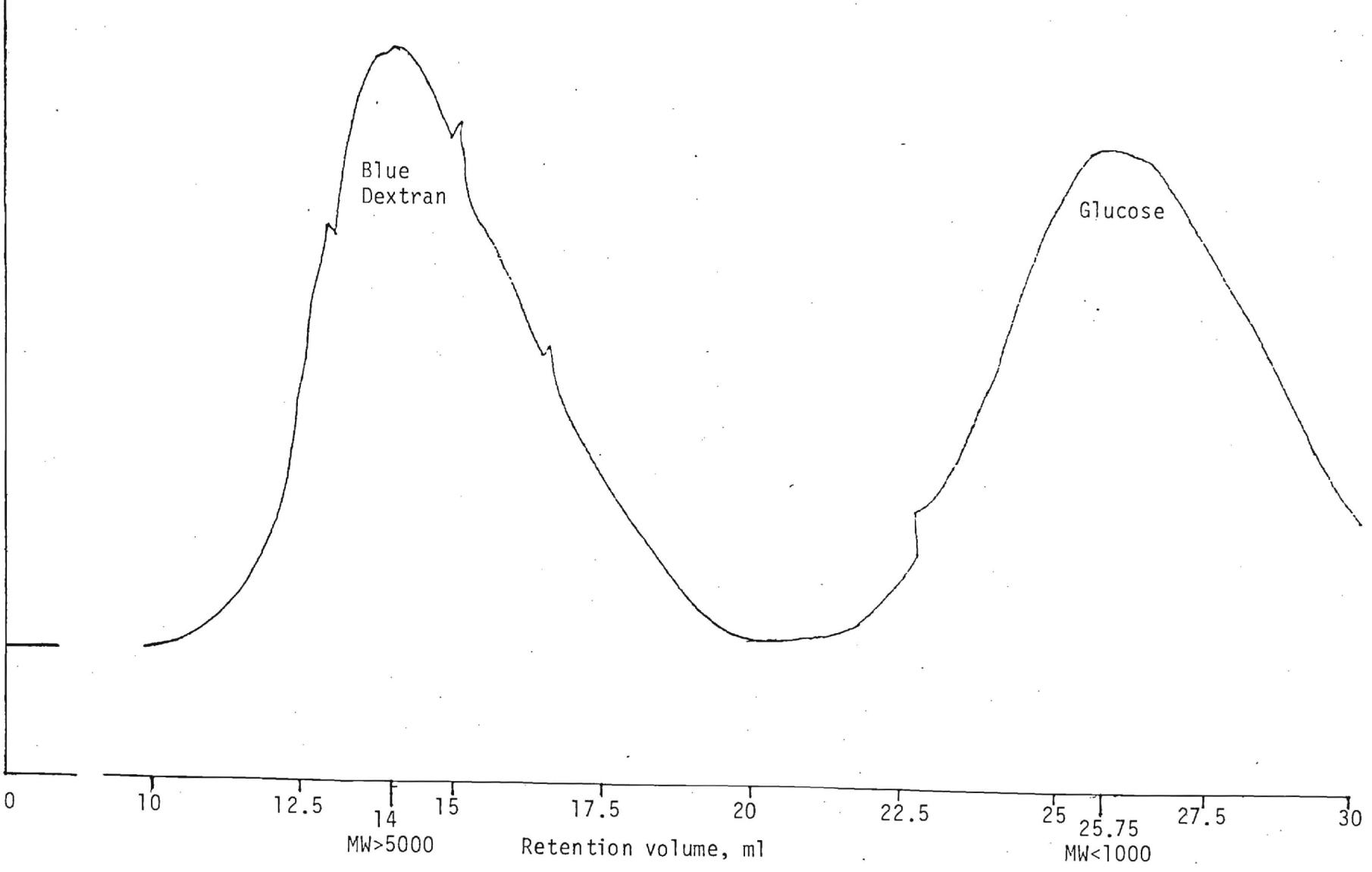


Figure 13. Fractionation range of Sephadex G25 column at a flow rate 20 ml/hr.

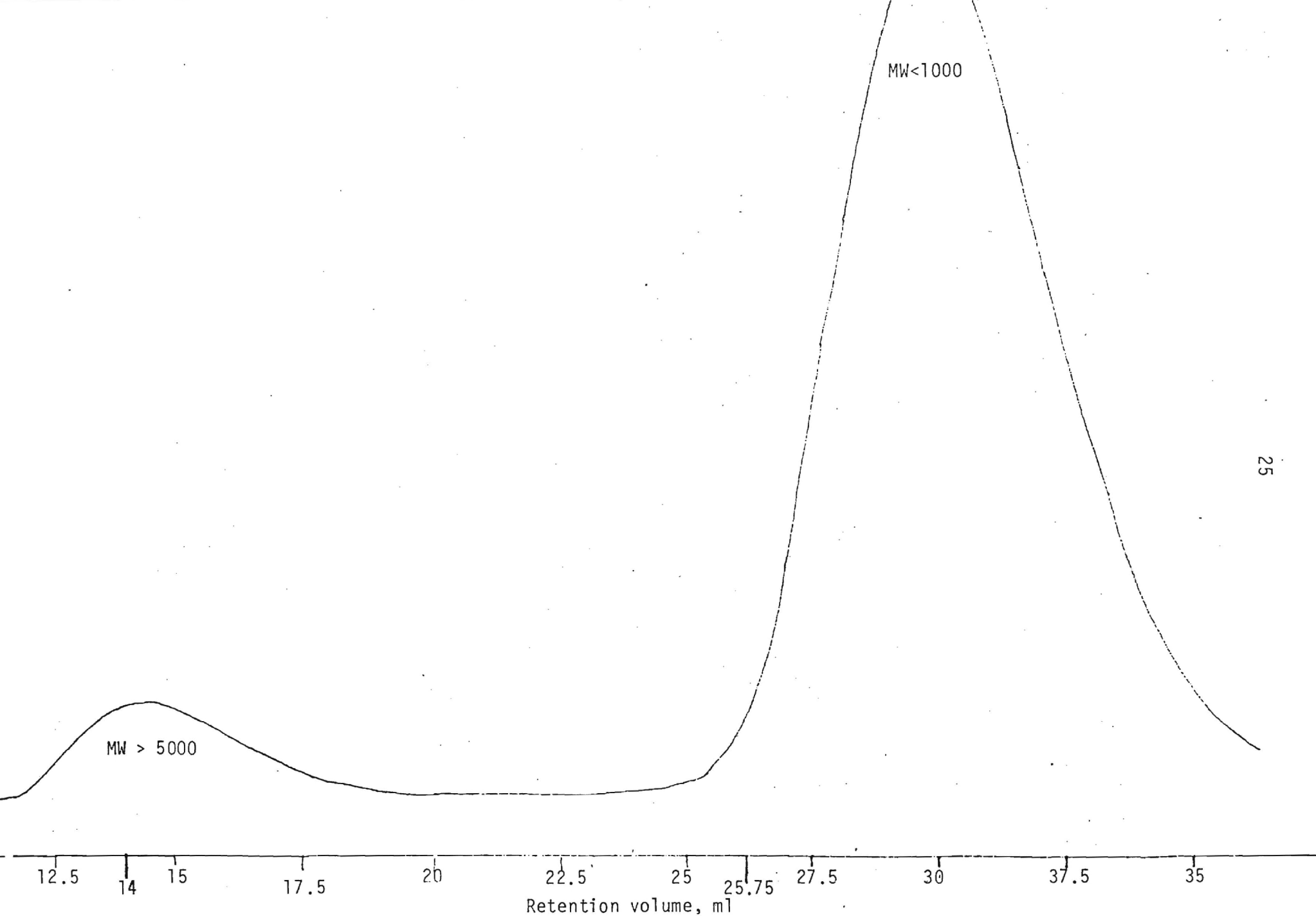


Figure 14. Fractionation of a riverwater humus on the Sephadex G25 column.
pH 4.5

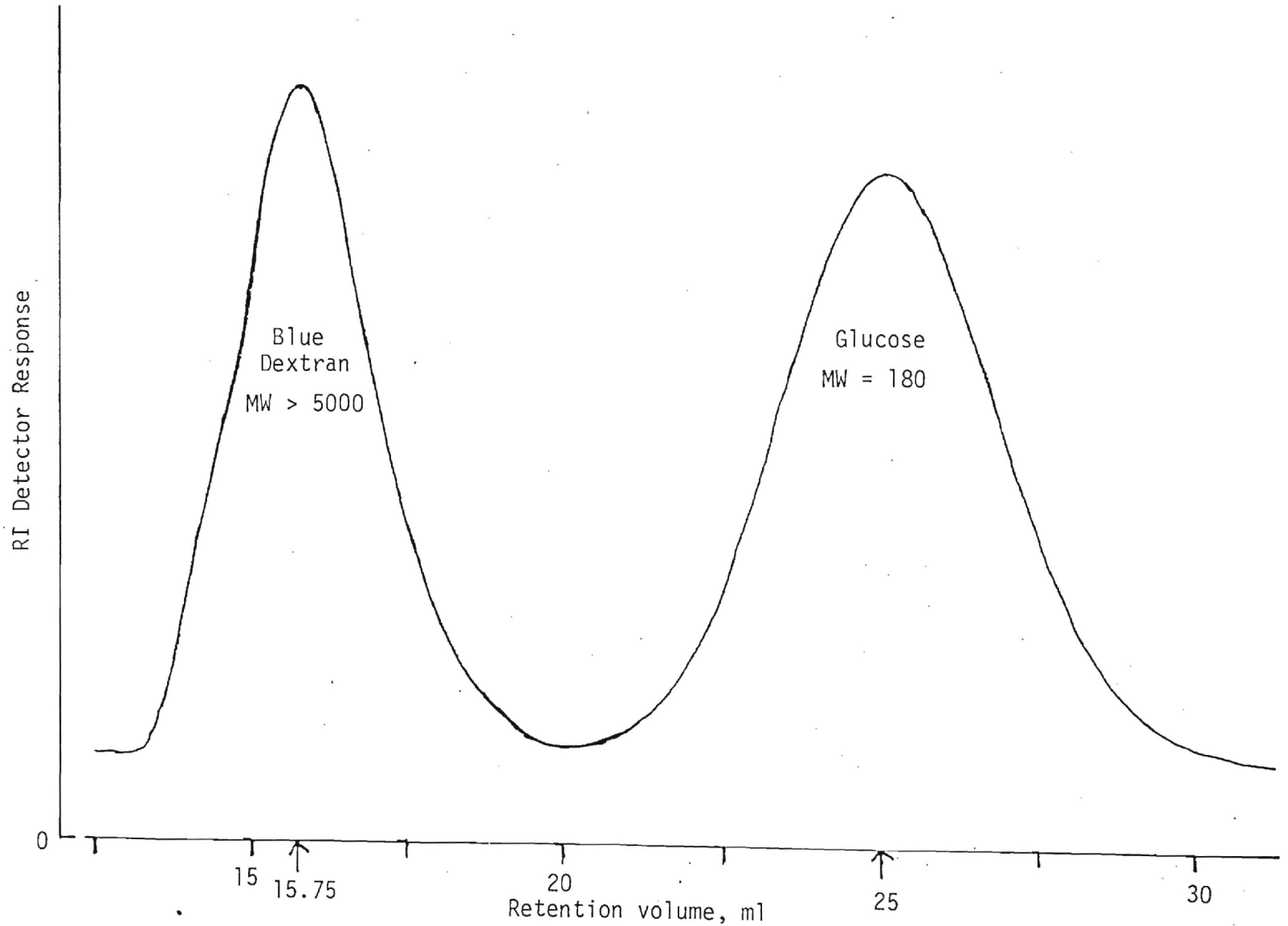
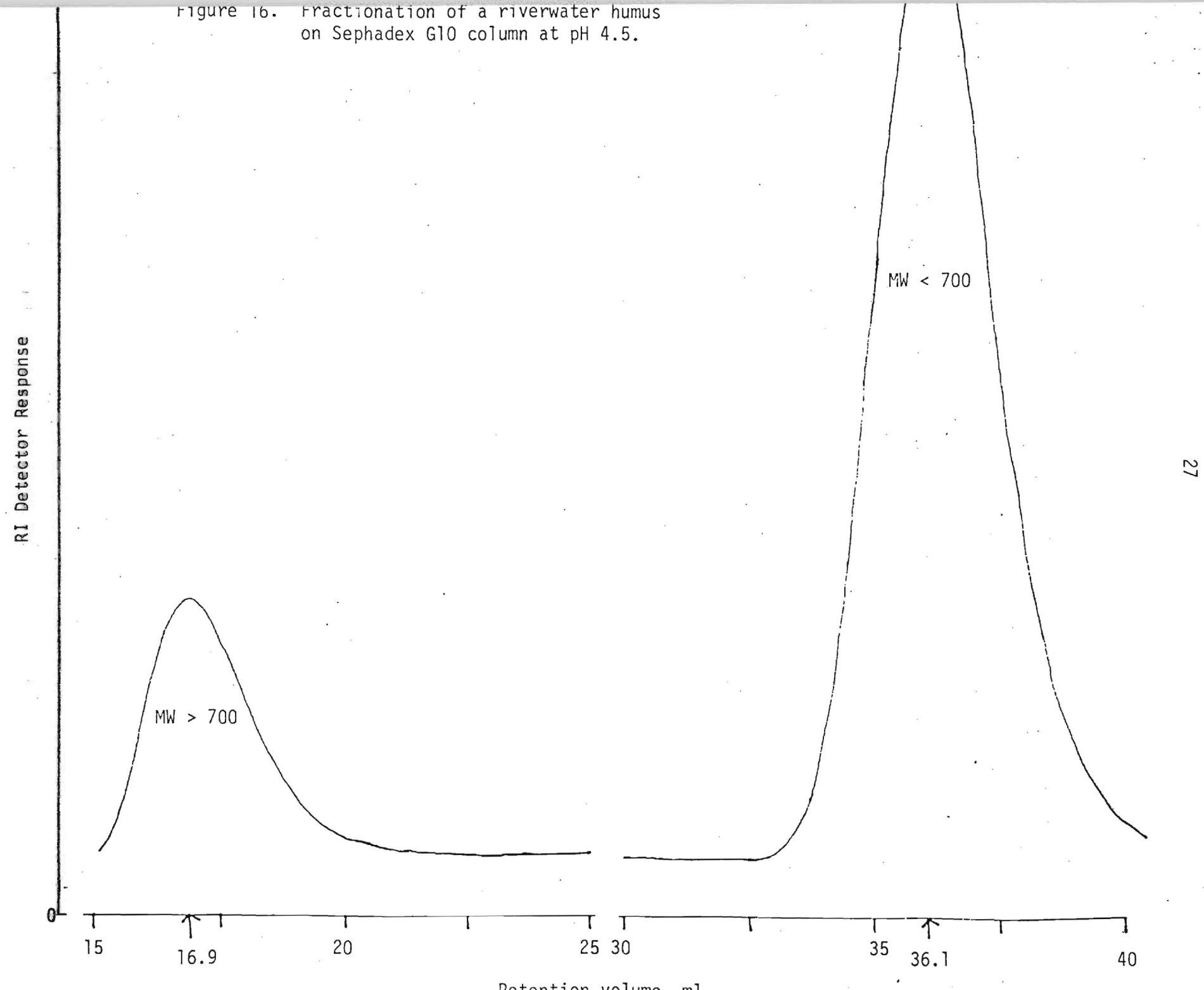


Figure 15. Fractionation range of Sephadex G10 column at a flow rate .23 ml/min.

Figure 16. Fractionation of a riverwater humus on Sephadex G10 column at pH 4.5.



An Amicon Model TCF-10 ultrafiltration (UF) unit with a UM50 filter installed (molecular weight cutoff of 500) was used to separate the humus into high and low molecular weight fractions. Before the UF system could be used to evaluate humus samples, the filter was tested for leaks (in terms of its molecular weight cutoff) using Blue Dextran 2000 (high molecular weight compound) and sodium chloride (low molecular weight compound). A Beckman Model 26 spectrophotometer was used to monitor the Blue Dextran concentration (with wavelength set at 619 nm). The NaCl was monitored with a Yellow Springs Instrument Model 31 Conductivity Bridge. The results indicated that there were no leaks. The unit was pressurized at 40 psig with N_2 and set at a recirculation rate of approximately 175 ml/min.

A 200 ml sample of a riverwater humus (M/39; 500 ppm soln) buffered at pH 6.8 and an ionic strength of 0.1M was ultrafiltered to 50 ml under the above conditions. The results are as follows:

	Vol	TOC
Initial	200 ml	309 ppm
Retentate	50 ml	892
Permeate	150 ml	44.5

$$\text{Recovered TOC} = 892\ (\tfrac{50}{200}) + 44.5\ (\tfrac{150}{200})\ \text{ppm}$$

$$= 256\ \text{ppm}$$

$$\%\ \text{Recovery} = 256/309 = 83$$

The Gel Permeation Chromatography (GPC) was performed using two gels: Sephadex G-25 and G-10 (Pharmacia Fine Chemicals) with fractionation ranges of 1000 to 5000 and 700 respectively. The gels were swollen overnight and the columns packed as recommended by the supplier. The elution volume of Blue Dextran and glucose were used to determine the molecular weight cuts from the GPC column with 4 fractions being collected. The results were as follows:

Mass balance after GPC

1 Fraction No	2 Vol	3 Measured TOC in ppm	4 Corrected TOC	5 Injection Volume	6 TOC in fraction 2 x 4÷5	
1	8.6ml	5.04	0	0.5ml	0	Background
2	9.6	66.24	61.20	0.5ml	1053	
3	6.9	6.48	1.44	0.5ml	20	
4	8.6	6.48	1.44	0.5ml	25	

Total	1098
Original	892
Balance	$\frac{1098}{892} \times 100 = 123\%$

96% of the material has a molecular weight >700 at pH 6.8. The unbuffered aqueous solution of the same material (pH 4.5) showed a molecular wt less than 700 on Sephadex G10 as well as G25 (Fig. 14 and 16). Figures 13 and 15 show the fractionation range of these columns.

The total organic carbon (TOC) content in the samples were monitored on a DC-52/54 Ultra low Carbon Analyzer (Dohrmann div. of Envirotech Ca.) Mircoliter amounts of the samples were injected in 10 ml of "organic free water" and analyzed for total organic carbon. A QA/QC for the calibration of the instrument was performed earlier. After establishing a water blank the calibration curve was rechecked with a 180 ppb standard. As the standard deviation for the standard fell within the 95% confidence interval at the reported level, the slope of the calibration curve was assumed same as the one established earlier. The samples were subsequently tested for their TOC content. The results were then corrected for the deviation in the value for the standard, and were reported in Table A.

6. Thermal gravimetric analysis

The instrument has been recently overhauled and checked by the manufacturers. Standard samples are being run to verify its working.

7. -COOH and phenolic -OH

These estimations need about 150-200 mg of sample. All samples with sufficient material will be run one after another.

SUMMARY

TASKS

No.	Sample name	Frn	UV	Fl	IR	El	X-ray	UF / SEC	TGA	Acid gr
1	MIAMI 1A									
2	MIAMI 1B	✓	✓	✓	✓	✓				
3	MIAMI 2									
4	N. ORLEANS Ia									
5	N. ORLEANS 2									
6	OTTUMWA I	✓	✓	✓	✓	✓				
7	OTTUMWA 2									
8	P2X									
9	PHILLY 1									
10	SEATTLE									

Frn = Fractionation
Fl = Fluorescence
El = Element analysis
TGA = Thermal gravimetric analysis
UF = Ultrafiltration
SEC = Size exclusion chromatography
Acid gr = analysis of phenolic and carboxyl groups.

The rest of the abbreviations are self-explanatory.

(✓) Check mark means the analysis read on the column is done for the sample named in the row.

FRACTIONATION

No.	Sample name	Weight fractionated	Wt. of fractions soluble in			Wt. of insoluble fraction
			Hexane	Ether	Acetone	
1	MIAMI 1A					
2	MIAMI 1B	1.04245	.04474	.35434	.49875	.00436
3	MIAMI 2					
4	N. ORLEANS Ia					
5	N. ORLEANS 2					
6	OTTUMWA I	1.07180	.02530	.19190	.19830	.07427
7	OTTUMWA 2					
8	P2X					
9	PHILLY 1					
10	SEATTLE					

Weights are in grams.

Ultraviolet absorption

No.	Sample name	Solvent	Concen. ppm	λ_{max} nm	$E^{1\%}_{1cm}$
0	AQUATIC HUMUS	H_2O	40 ppm	268	82.5
1	MIAMI 1A				
2	MIAMI 1B	MeOH	61.4	208	171
3	MIAMI 2				
4	N. ORLEANS Ia				
5	N. ORLEANS 2				
6	OTTUMWA I	MeOH	44.2	210	215
7	OTTUMWA 2				
8	P2X				
9	PHILLY 1				
10	SEATTLE				

Fluorescence

No.	Sample name	Solvent	Concen. ppm	λ_{max} nm	
				Ex	Em
0	AQUATIC HUMUS	H_2O	40	460	365
1	MIAMI 1A				
2	MIAMI 1B	MeOH	61.4	370	463
3	MIAMI 2				
4	N. ORLEANS Ia				
5	N. ORLEANS 2				
6	OTTUMWA I	MeOH	44.2	348	417
7	OTTUMWA 2				
8	P2X				
9	PHILLY 1				
10	SEATTLE				

Element analysis

No.	Sample name	C	H	N	S	Cl	Br	Halogen	Ash
0	AQUATIC HUMUS								
1	MIAMI 1A								
2	MIAMI 1B	57.51	6.11	2.27	2.07	1.45	0.24		0.91
3	MIAMI 2								
4	N. ORLEANS Ia								
5	N. ORLEANS 2								
6	OTTUMWA I	58.31	6.73	2.95	0.94	0.30	<.05		0.46
7	OTTUMWA 2								
8	P2X								
9	PHILLY 1								
10	SEATTLE								

Future plan

In accordance with the telephone conversation with the EPA project officer, the analytical procedures are being listed according to their priorities based on the quantity of sample available.

1. UV, Fluorescence and IR spectra
2. Element analysis
3. X-ray analysis
4. Ultrafiltration/Size exclusion chromatography
5. Thermal gravimetric analysis
6. Analysis of phenolic and carboxyl groups.

The following analyses will be completed during the following quarter: (i) All fractionations and analyses 1 and 2, (ii) X-ray scattering analysis will be done near pH 11, 7 and 4 in this sequence of priority according to the quantity of material available. If very little material of a sample is available, the analysis will be done at pH 11 only, because it appears that the molecules aggregate at lower pH. The most suitable pH condition of the SEC/UF appears to be pH 6.8 in spite of the x-ray scattering results, because that is close to the pH encountered in drinking water. SEC/UF procedure to be followed will be finally decided after we have data on the weights of acetone soluble fractions of the samples. (iii) TGA (analysis 5) will be done. (iv) Acidic functional group analysis requires the largest amount of samples which will be determined only after all the other analyses are done.

E-20-616

COMPARISON OF HIGH MOLECULAR WEIGHT ORGANICS ISOLATED FROM DRINKING WATER IN FIVE CITIES

QUARTERLY REPORT
April 15, 1982

by

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Project Officer
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TASKS

No.	Sample name	Frn	UV	Fl	IR	El	X-ray	UF / SEC	TGA	Acid gr
1	MIAMI 1A	✓	✓	✓	✓					
2	MIAMI 1B	✓	✓	✓	✓	✓	✓			
3	MIAMI 2	✓	✓	✓	✓					
4	N. ORLEANS Ia	✓	✓	✓	✓					
5	N. ORLEANS 2	✓	✓	✓	✓					
6	OTTUMWA 2	✓	✓	✓	✓	✓	✓			
7	OTTUMWA 1	✓	✓	✓	✓					
8	P2X	✓	✓	✓	✓					
9	PHILLY 1	✓	✓	✓	✓					
10	SEATTLE	✓	✓	✓	✓					

Frn = Fractionation

Fl = Fluorescence

El = Element analysis

TGA = Thermal gravimetric analysis

UF = Ultrafiltration

SEC = Size exclusion chromatography

Acid gr = analysis of phenolic and carboxyl groups.

The rest of the abbreviations are self-explanatory.

(✓) Check mark means the analysis read on the column is done for the sample named in the row.

FRACTIONATION

No.	Sample name	Weight fractionated	Wt. of fractions soluble in			Wt. of insoluble fraction
			Hexane	Ether	Acetone	
1	MIAMI 1A	2.0595	.0740	.5943	1.0115	.0148
2	MIAMI 1B	1.0424	.0447	.3637	.5111	.0044
3	MIAMI 2	4.8950	.0889	1.1041	1.0903	.0279
4	N. ORLEANS Ia	1.8828	.0324	.6434	.5221	.0897
5	N. ORLEANS 2	2.6387	.0418	.6717	.5298	.1734
6	OTTUMWA 2	2.3787	.0523	.4138	.4425	.1734
7	OTTUMWA 1	2.0784	.0305	.2544	.8483	.1202
8	P2X	1.5244	.0280	.4808	.4575	.0469
9	PHILLY 1	4.5167	.0509	.7937	1.1308	.0595
10	SEATTLE	.5007	.0065	.0859	.0344	.0259

Weights are in grams.

Ultraviolet absorption

No.	Sample name	Solvent	Concen. ppm	λ_{max} nm	$E^{1\%}_{1cm}$
0	AQUATIC HUMUS	H_2O	40	268	82.5
1	MIAMI 1A	MeOH	40	212	242
2	MIAMI 1B	MeOH	61	208	171
3	MIAMI 2	MeOH	55	214	244
4	N. ORLEANS Ia	MeOH	40	210	202
5	N. ORLEANS 2	MeOH	60	213	215
6	OTTUMWA 2	MeOH	44	210	215
7	OTTUMWA 1	MeOH	44	208	150
8	P2X	MeOH	46	210	221
9	PHILLY 1	MeOH	44	212	227
10	SEATTLE	MeOH	38	208	179

Fluorescence

No.	Sample name	Solvent	Concen. ppm	λ_{max} nm	
				Ex	Em
0	AQUATIC HUMUS	H_2O	40	365	460
1	MIAMI 1A	MeOH	40	355	425
2	MIAMI 1B	MeOH	61	370	463
3	MIAMI 2	MeOH	55	352	426
4	N. ORLEANS Ia	MeOH	40	355	425
5	N. ORLEANS 2	MeOH	60	348	426
6	OTTUMWA 2	MeOH	44	348	417
7	OTTUMWA 1	MeOH	44	355	420
8	P2X	MeOH	46	350	430
9	PHILLY 1	MeOH	44	346	426
10	SEATTLE	MeOH	38	350	430

Infrared Spectra

No.	Name	Important Absorptions OH	C=O
1	Miami 1A	3400 cm^{-1}	1720 cm^{-1}
2	Miami 1B	3440-3380	1720-1695
3	Miami 2	3400	1720-1690
4	N. Orleans 1a	3420-3360	1705
5	N. Orleans 2	3390	1720-1710
6	Ottumwa 2	3390	1725,1700
7	Ottumwa 1	3360	1710
8	P2X	3420-3360	1710
9	Philly 1	3400	1695
10	Seattle	3420	1710

X-ray Scattering

No.	Name	Rg (Å)	pH	Concn.	Comments
1	Miami 1A				
2	Miami 1B	7.6±0.2	9	1%	Polydisperse
3	Miami 2				
4	N. Orleans 1a				
5	N. Orleans 2				
6	Ottumwa 2	5.4±0.5	10	1%	Monodisperse
7	Ottumwa 1				
8	P2X				
9	Philly 1				
10	Seattle				

Rg = Radius of gyration

COMPARISON OF HIGH MOLECULAR WEIGHT ORGANICS ISOLATED FROM DRINKING WATER IN FIVE CITIES

Final Report

by

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COMPARISON OF HIGH MOLECULAR WEIGHT ORGANICS ISOLATED FROM DRINKING WATER IN FIVE CITIES

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Experimental

Ten samples of organic matter isolated from drinking water of five cities and two samples of chlorinated fluka humus (CFH) were provided by the Health Effects Research Laboratory (HERL/EPA, Cincinnati, Ohio). A sample of aquatic humic substances isolated from the Satilla river was used for comparison. A list of the samples is presented in Table 1. Each sample was fractionated according to a prescribed procedure (1). Organic solvents used for the fractionation were all "distilled in glass" grade (Burdick & Jackson, Muskegon, MI). The acetone soluble fraction of each sample was subjected to the following analyses.

Elemental Analysis

The fractions were analyzed for C, H, and N by an automatic analyzer. Sulfur and the halogens (i.e., Cl and Br) were analyzed by combustion and subsequent titration. The following standard compounds were used for quality assurance (QA) purposes:

Acetanilide	C,H,N
Sulfanilamide	S
p-Chlorobenzoic acid	Cl
p-Bromobenzoic acid	Br

Spectral Studies

UV spectra were recorded in methanolic solution (40-60ppm) on a Beckman Model 26 spectrophotometer (Irvine, CA), using matched quartz cells of 1-cm path length. A holmium oxide spectrum was recorded prior to the analysis of the samples for QA purposes.

Infrared spectra were recorded using a Beckman Acculab 6 spectrophotometer (Fullerton, CA). Pellets were prepared by torque pressing of a

Table 1. List of samples investigated in this study

No.	Name	Detailed Descriptions
1	Miami 1A	
2	Miami 1B	
3	Miami 2	
4	New Orleans 1A	
5	New Orleans 2	
6	Ottumwa 2	
7	Ottumwa 1	
8	P2X	
9	Philly	
10	Seattle	
11	CFH-1	Low pH chlorination, 1 g/L 1:1 ratio 1 of 2, Cl-Fluka humics of 1982. One liter lyophilized to dryness on 1/26/83. Tare wt. 22.76100 Humic wt. 5.946 Bright brown dry solid
12	CFH-2	Low pH chlorination, 1 g/L 1:1 ratio 2 of 2, Cl-Fluka humics of 1982. One liter lyophilized to dryness on 1/26/83. Bright brown dry solid

finely ground mixture composed of 1-2 mg of sample and 100-500 mg of spectroanalytical KBr. The pellets were translucent brown and showed good dispersion of the sample. Considerable attention was given to prevent moisture interference. The spectrum of a polystyrene film was recorded prior to the sample for QA purposes.

Fluorescense spectra were recorded in methanolic solution (40-60 ppm) on a Perkin-Elmer Model/204A spectrofluorometer (Norwalk, CT). Mutually maximizing excitation and emission wavelengths were recorded in nm units. Water was used for QA purposes.

X-Ray Scattering

Each sample was analyzed by a low angle x-ray scattering technique outlined by Wershaw and Pinkey (2).

Ultrafiltration (UF) and Size Exclusion Chromatography (SEC)

The apparent molecular weight distribution of each sample was determined by sequential use of UF and SEC which was modified from a procedure proposed by Chian and De Walle (3). Approximately 10 mg of a sample was dissolved in 200 ml of an alkaline buffer solution (pH = 10.4; 12.1 g of tris(hydroxy-methyl)aminomethane per liter of "organic free" water). A UM05 UF membrane (Amicon, Burlington, MA) having a molecular weight cut-off at 500 Dalton was used in a static test cell at a nitrogen pressure of 35 psi. Fifty ml of retentate of each sample were collected. The UF retentate was further fractionated by SEC on Sephadex G-75 (100 x 1-cm I.D. glass column, bed height 41 cm) (Pharmacia Fine Chemicals, Piscataway, NJ). The flow rate was maintained at 18 ml/h by means of a peristaltic pump (Milton Roy) and the column effluent was monitored by a variable UV detector (Perkin-Elmer, Norwalk, CT). The exclusion volumes of the get permeation column were found by chromatographing a mixture of blue-dextran (MW 2,000,000 Dalton) and phenol, and monitoring the

column effluent with a variable UV detector (model LC-65T, Perkin-Elmer, Norwalk, CT, at 230 nm.

Thermal Gravimetric Analysis (TGA) and Differential Thermal Analysis (DTA).

Approximately 10 mg of accurately weighed sample were subjected to TGA and DTA. The percentage weight loss under a temperature program of 25°C/min was automatically recorded on a Mettler Thermoanalyzer 2 and on a Perkin-Elmer TGS-2 Thermogravimetric System (Norwalk, CT). The mean weight loss of a $CaCo_3$ standard (3 runs) was 42.9% (theoretical 44%) with a precision of $\pm$ 1.0%. The minimum weight of 10 mg required for the analysis was determined by a test with aquatic humus on TGA.

Analysis of Acidic Functional Groups

Carboxyl groups and total acidity (TA) of the samples were determined as follows:

a. Carboxyl Group Analysis

To an accurately weighed sample (circa 10 mg) in a 5 ml vial it were added 3 ml of CO_2 free water and 0.2 ml of 0.1 N NaOH. The mixture was stirred with a micro magnetic stir bar until the solution was complete. The mixture was then added to a freshly prepared 0.2N CH_3COONa solution (100ml), whose pH was recorded beforehand (pH_o). Three aliquots of CO_2 free water (total volume 1 ml) were used to wash the vial and the washings were added to the bulk solution. 0.2 ml of 0.1 N HCl was added to neutralize the alkali initially used and the pH of this mixture was measured. The mixture was titrated back to pH_o with 0.1 NaOH using a microburette. Carboxyl group concentration was calculated as follows:

$$[COOH] = \frac{x(V-V_o)}{w/1000} \text{ meq/g,}$$

where x = strength of NaOH (N)

v = volume (ml) of NaOH consumed for sample

V_o = volume (ml) of NaOH consumed for blank

W = weight of sample (mg)

Eleven analyses of an aquatic humus sample gave a mean value of 4.76 meq/g with a standard deviation (s) of 0.14 and a coefficient of variation (cv) of 2.9%.

[COOH] values for the same sample by the procedure proposed by Perdue et al. (4) gave a mean of 4.27 meq/g with a s = 0.08 and a cv = 1.9%.

b. Total Acidity (TA)

The procedure proposed by Schnitzer and Gupta (5) and Schnitzer and Kahn (6) was adopted with some modification for the determination of TA. All operations were carried out under N_2 atmosphere. Approximately, 10 mg of accurately weighed samples were used for this determination. A blank experiment and six repetitive analyses from the Satilla river were performed. The mean value was 11.1 meq/g with s = 0.35 and cv = 3.2%.

Results and Discussion

The results of the fractionation of the samples according to their solubility in hexane, ether and acetone are presented in Table 2. The acetone fractions of the Samples vary between 22.5 and 67.7% of the total recovered weight; the ether fractions range between 20.3 and 56.2%. The two chlorinated Fluka-Humus (CFH) samples gave a consistently lower percentage for these two fractions. Further characterization of the acetone soluble fractions is described below

Elemental Analysis

The elemental analyses are presented in Table 3. The atomic ratios H/C for all samples are between 1.3 and 1.4. This is comparable to humic acid derived from lake sediments. However, N/C ratios are much lower when compared to those humic substances. Br is present in almost negligible quantities, whereas Cl varies between 0.3 and 2.2% and S between 0.9 and 2.7%. All fractions from drinking water show similar elemental composition. However, they differ from the elemental composition of the CFH samples.

Spectral Studies

a. Ultraviolet Absorption

The acetone fractions derived from drinking water exhibit intense UV absorption between 208 and 214 nm, which may be attributed to unsaturated acids or esters. The CFH samples presented maximum absorption at 254-256 nm, whereas a aquatic humus sample derived from the Satilla river showed a maximum absorption at 234 nm.

b. Fluorescense Spectra

Fluorescence data are presented in Table 4. All samples derived from drinking water, gave an emission maximum between 417 and 430 nm, while the

Table 2. Fractionation of samples under investigation

	Sample	Initial Weight Fractionated	Fraction Soluble in			Insoluble Fraction	Percentage Recovered from Total Amount Fractionated
			Hexane	Ether	Acetone		
1	Miami 1A	2.0595	.0740 (4.4)	.5943 (35.1)	1.0115 (59.6)	.0148 (0.9)	82.2
2	Miami 1B	1.0424	.0447 (4.8)	.3637 (39.4)	.5111 (55.3)	.0044 (0.5)	88.6
3	Miami 2	4.8950	.0839 (3.8)	1.1041 (47.8)	1.0903 (47.2)	.0279 (1.2)	47.2
4	New Orleans 1A	1.8828	.0324 (2.5)	.6434 (50.0)	.5221 (40.5)	.0897 (7.0)	68.4
5	New Orleans 2	2.6387	.0418 (2.8)	.6711 (44.3)	.6298 (41.5)	.1734 (11.4)	57.5
6	Ottumwa 2	2.3787	.0523 (4.8)	.4138 (38.2)	.4425 (40.9)	.1734 (16.1)	45.5
7	Ottumwa 1	2.0784	.0305 (2.4)	.2544 (20.3)	.8483 (67.7)	.1202 (9.6)	60.3
8	Philly 2x	1.5244	.0280 (2.7)	.4808 (47.4)	.4575 (45.1)	.0469 (4.6)	66.4
9	Philly 1	4.5167	.0509 (2.5)	.7937 (39.0)	1.1308 (55.5)	.0595 (2.9)	45.1
10	Seattle	.5007	.0065 (4.3)	.0859 (56.2)	.0344 (22.5)	.0259 (17.0)	30.5
11	CFH-1	1.0268	.0042 (0.4)	.0387 (3.9)	.1212 (12.3)	.8244 (83.4)	96.3
12	CFH-2	1.0524	.0043 (0.4)	.0415 (4.3)	.1225 (12.8)	.7922 (82.5)	91.3

Weight are in grams

* ( ) Percentage of each fraction with respect to total amount recovered

Table 3. Elemental analysis of acetone soluble fractions (%).

No.	Sample name	C	H	N	S	Cl	Br	Ash
1	Miami 1A	54.41	6.04	1.74	2.74	2.19	0.39	0.54
2	Miami 1B	57.51	6.11	2.27	2.07	1.45	0.24	0.91
3	Miami 2	57.20	6.19	1.85	1.13	1.42	0.36	1.06
4	N. Orleans 1A	56.11	6.50	1.47	0.87	2.24	0.14	1.91
5	N. Orleans 2	55.58	6.41	1.74	0.92	1.35	0.23	0.92
6	Ottumwa 2	58.31	6.73	2.95	0.94	0.30	<.10	0.46
7	Ottumwa 1	55.77	6.13	2.56	0.93	0.57	0.11	0.34
8	P2X	53.67	6.13	1.99	1.88	1.56	0.20	0.45
9	Philly 1	55.80	6.19	1.77	1.50	1.26	0.22	0.43
10	Seattle	52.83	6.03	2.11	1.04	2.02	<.10	0.97
11	CFH1	41.27	3.70	0.53	2.43	14.61	32.94	2.51
12	CFH2	39.34	3.41	0.53	2.40	16.08	36.25	2.80

Table 4. Fluorescence spectra of acetone soluble fractions

No.	Sample Name	Solvent	λ_{max} nm	
			Ex	Em
0	Aquatic Humus	MeOH	335	450
1	Miami 1A	MeOH	355	425
2	Miami 1B	MeOH	370	463
3	Miami 2	MeOH	352	426
4	N. Orleans 1A	MeOH	355	425
5	N. Orleans 2	MeOH	348	426
6	Ottumwa 2	MeOH	348	417
7	Ottumwa 1	MeOH	355	420
8	P2X	MeOH	350	430
9	Philly	MeOH	346	426
10	Seattle	MeOH	350	430
11	CFH1	MeOH	370	450
12	CFH2	MeOH	370	460

excitation maximum ranged between 346 and 355 nm. Miami 1B showed an excitation maximum similar to the CFH samples, but quite different from the one exhibited by the aquatic humic substances from the Satilla river. The emission maximum of these samples (See Table 4) however, was very similar.

c. Infrared Spectra

The IR spectra of all ten samples derived from drinking water are almost identical. Figure 1 shows a representative spectrum. For comparison the IR spectra of Satilla river aquatic humus and CFH are added. All samples exhibit broad bands in the ~3400 cm^{-1} (-OH), ~1720 cm^{-1} (C=O) and 1640 cm^{-1} (C=C) regions. However, the spectra from the ten drinking water samples and CFH samples show distinct differences from that of the Satilla river aquatic humus which absorbs sharply near 1600 cm^{-1}. This may suggest that the samples derived from drinking water are strongly altered by chlorination and/or oxidation. Further confirmation of the spectral dissimilarities should be pursued by analysis of chlorinated alteration products of humus.

X-Ray Scattering

The results of the low angle x-ray scattering analyses are reported in Table 5.

Ultrafiltration and Size Exclusion Chromatography

The UF separation (i.e. drinking water organic matter, CFH) gave similar values for all samples. 89% or more of the initial weight was recovered in the retentate fraction (See Table 6.) The subsequent analysis of the UF retentate by Sephadex G-75 showed differences in molecular weight distribution of the two samples categories. The typical elution patterns are shown in Figure 2, along with the calibration standard. The apparent molecular weights of drinking water derived samples range between 1000 and 500 Dalton, whereas the CFH samples present a molecular weight ~5000 Dalton.

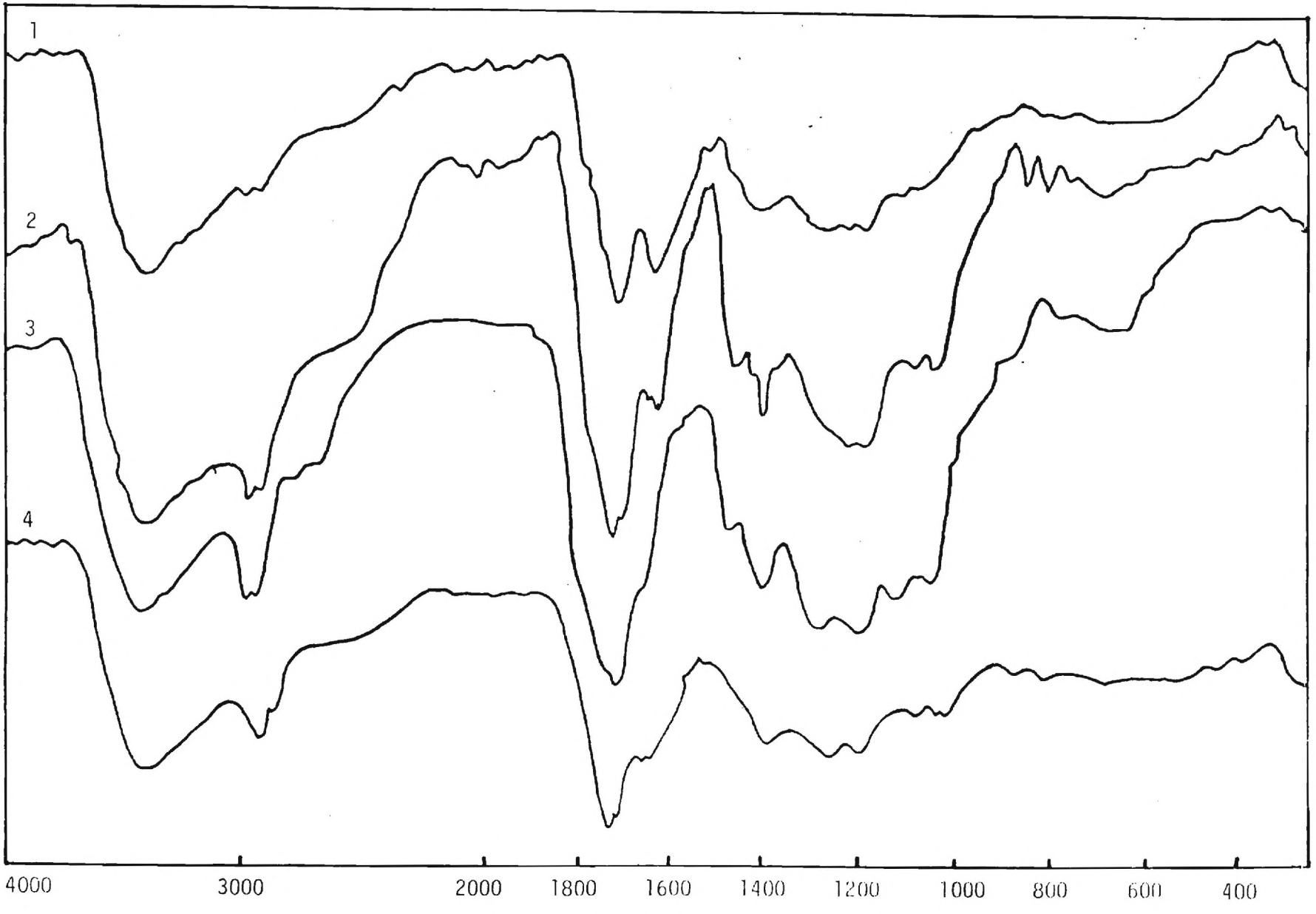

Figure 1. Infrared Spectra of: 1. Aquatic River Humus; 2. Chlorinated Aquatic River Humus; 3. Organics from Drinking Water (Philly 1, #9), and 4. Chlorinated Fluka Humus (CFH)

Table 5. X-ray scattering of acetone soluble fraction

No.	Name	R_g	pH
1	Miami 1A	5.6	11
2	Miami 1B	7.6	9
3	Miami 2	7.4	11
4	New Orleans 1A	6.4	11
5	New Orleans 2	8.4	11
6	Ottumwa 2	5.4	10
7	Ottumwa 1	5.3	11
8	P2X	7.2	11
9	Philly	10.9	11
10	Seattle	Not done.	Insufficient quantity.
11	CFH 1	8.7	11
12	CFH 2	6.8	11

Table 6. Ultrafiltration of acetone soluble fractions

No.	Sample	Original Wt (mg)	Retentate Wt (mg)	Retentate % Recovery	Permeate Wt (mg)	Permeate % Recovery	Total Recovery
1	Miami 1A	10.1	9.6	95	0	0	95
2	Miami 1B	10.3	12.1	117	0.3	3.6	120
3	Miami 2	10.1	9.9	98	1.8	18.1	116
4	New Orleans 1A	9.2	9.2	89	0	0	89
5	New Orleans 2	9.2	10.9	119	2.0	22.0	140
6	Ottumwa 2	9.9	9.1	92	1.0	9.7	102
7	Ottumwa 1	9.9	10.6	107	0	0	107
8	P2X	9.7	10.4	107	1.6	16.0	124
9	Philly 1	9.6	9.0	94	1.0	10.9	104
10	Seattle	Not done.	Insufficient quantity				
11	CFH-1	11.0	11.1	101	0.6	5.5	107
12	CFH 2	11.0	11.1	101	0.6	5.5	107

Volume of original solution = 200 ml
Volume of retentate = 50 ml
Volume of permeate = 150 ml

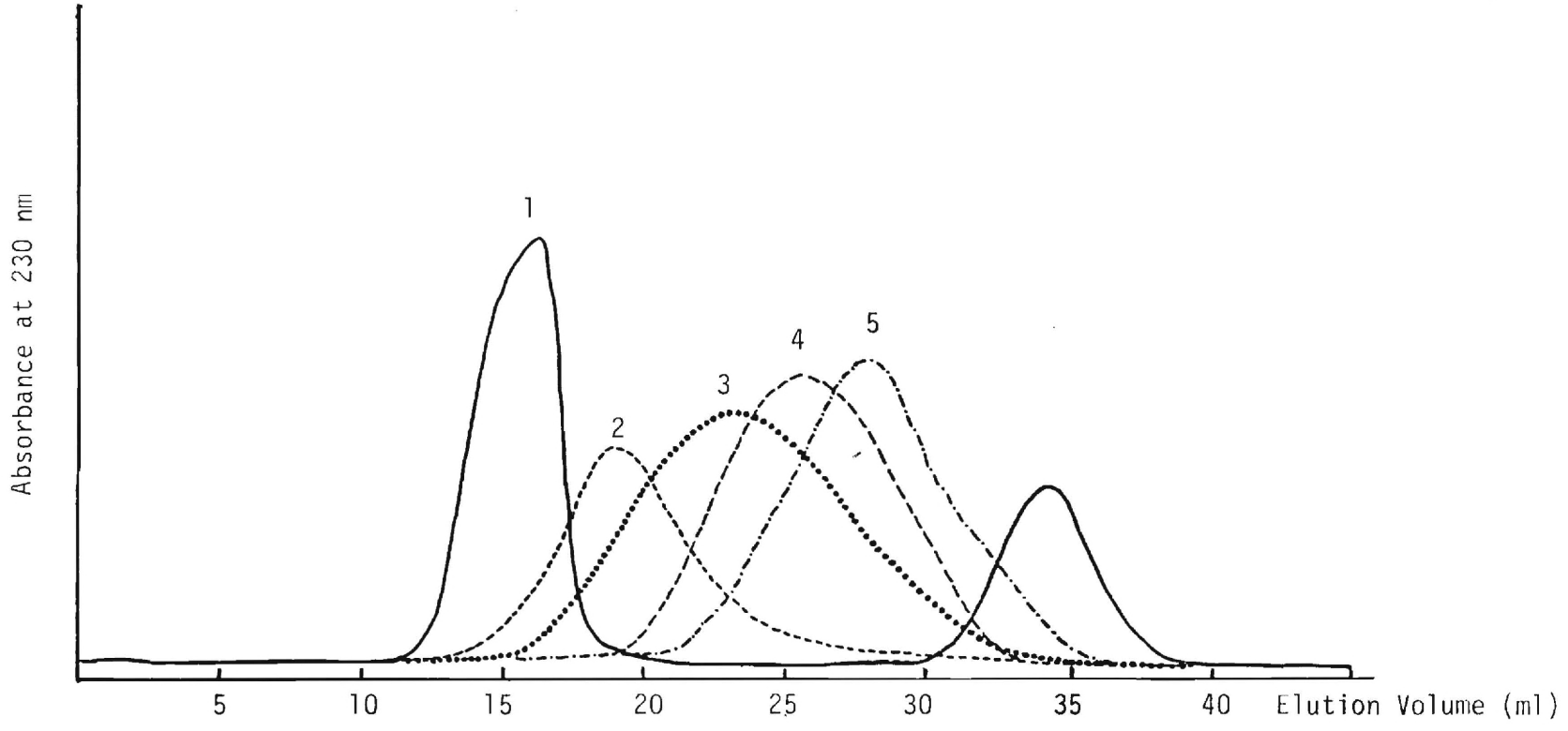

Figure 2. Gel Permeation Chromatography on Sephadex G75 of: 1. Blue Dextran and Phenol Standard; 2. Aquatic River Humus; 3. Chlorinated Fluka Humus (CFH); 4. Chlorinated Aquatic River Humus; and 5. Organics from Drinking Water (Philly 1, #9).

TGA and DTA

TGA and DTA of the samples derived from drinking water gave similar thermal maxima temperatures (thermal maxima temperatures were within 20°C) and thermal maxima curves. On the other hand, the CFH samples, although similar within themselves, presented widely different thermal maxima temperatures and curve shapes when compared with the ten samples derived from drinking water. Representative DTA curves for these two sample categories are shown in Figure 3.

Acidic Group Analyses

[COOH] and total acidity values are reported in Table 7. Two distinct groupings can be recognized according to the two sample categories, although one CFH sample presents a [COOH] value (i.e., CFH2) similar to at least three values found for the drinking water samples.

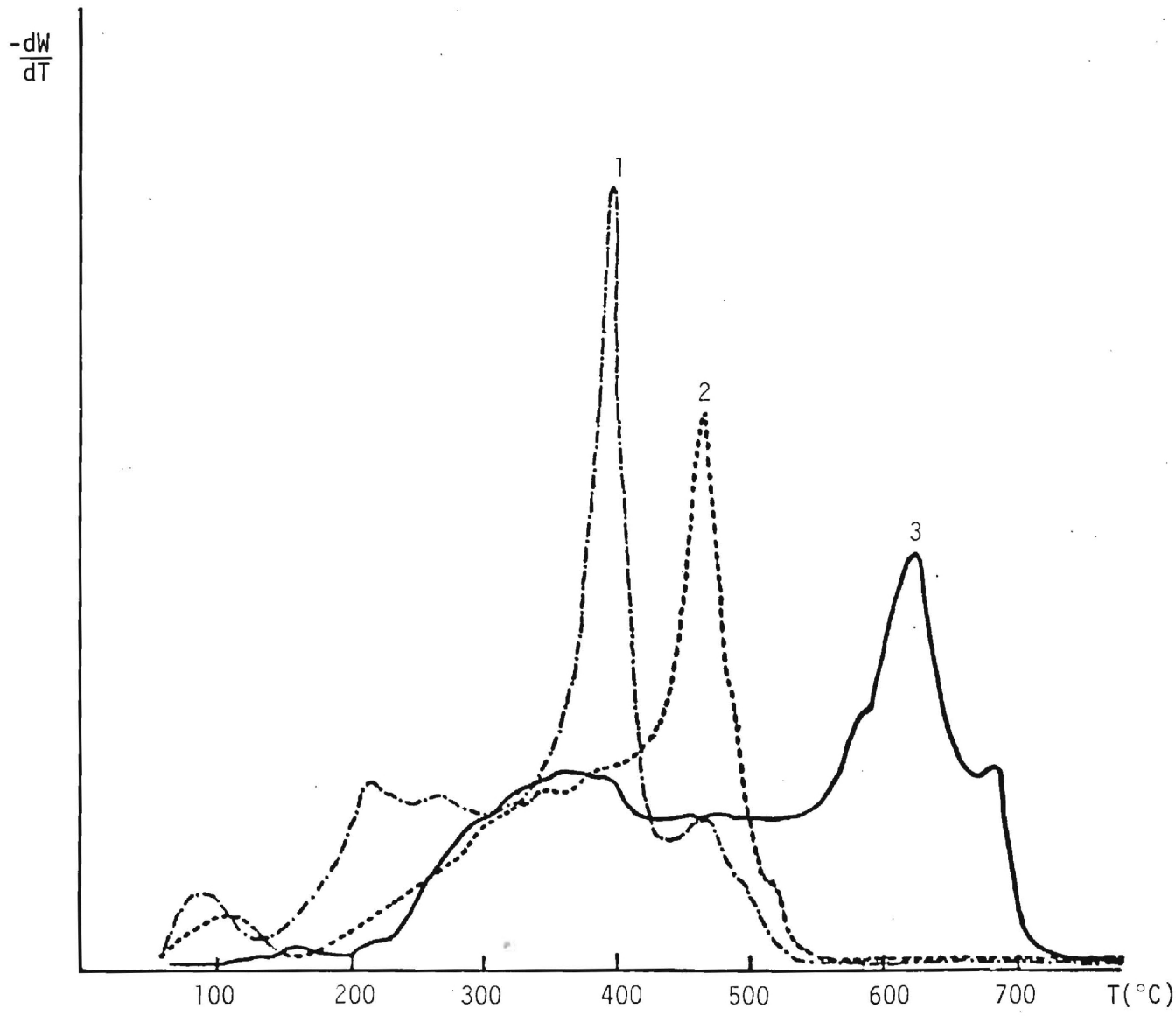

Figure 3. Differential Thermogravimetric Curves of: 1. Chlorinated Fluka Humus; 2. Aquatic River Humus; and 3. Organics from Drinking Water (Ottumwa 1).

Table 7. Acidic functions of acetone soluble fraction

No.	Name	COOH meq/g	Total Acidity meq/g
1	Miami 1A	2.5	5.9
2	Miami 1B	2.4	6.1
3	Miami 2	2.7	5.8
4	New Orleans 1A	1.9	7.0
5	New Orleans 2	2.0	5.6
6	Ottumwa 2	2.3	5.3
7	Ottumwa 1	2.7	7.3
8	P2X	2.3	5.2
9	Philly 1	1.9	7.4
10	Seattle	Not done.	Insufficient quantity.
11	CFH 1	3.1	3.8
12	CFH 2	2.6	3.4

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